Mode of Action of Liver Tumor Induction by Trichloroethylene and Its Metabolites, Trichloroacetate and Dichloroacetate

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Trichloroethylene (TCE) induces liver cancer in mice but not in rats. Three metabolites of TCE may contribute—chloral hydrate (CH), dichloroacetate (DCA), and trichloroacetate (TCA). CH and TCA appear capable of only inducing liver tumors in mice, but DCA is active in rats as well. The concentrations of TCA in blood required to induce liver cancer approach the mM range. Concentrations of DCA in blood associated with carcinogenesis are in the sub-μM range. The carcinogenic activity of CH is largely dependent on its conversion to TCA and/or DCA. TCA is a peroxisome proliferator in the same dose range that induces liver cancer. Mice with targeted disruptions of the peroxisome proliferator-activated receptor alpha (PPARα) are insensitive to the liver cancer-inducing properties of other peroxisome proliferators. Human cells do not display the responses associated with PPARα that are observed in rodents. This may be attributed to lower levels of expressed PPARα in human liver. DCA treatment produces liver tumors with a different phenotype than TCA. Its tumorigenic effects are closely associated with differential effects on cell replication rates in tumors, normal hepatocytes, and suppression of apoptosis. Growth of DCA-induced tumors has been shown to arrest after cessation of treatment. The DCA and TCA adequately account for the hepatocarcinogenic responses to TCE. Low-level exposure to TCE is not likely to induce liver cancer in humans. Higher exposures to TCE could affect sensitive populations. Sensitivity could be based on different metabolic capacities for TCE or its metabolites or result from certain chronic diseases that have a genetic basis. *Key words*: chloral hydrate, dichloroacetate, liver tumors, mode of action, trichloroacetate, trichloroethylene. — *Environ Health Perspect* 108(suppl 2):241–259 (2000). *http://ehpnet1.niehs.nih.gov/docs/2000/suppl-2/241-259bull/abstract.html*

Trichloroethene (TCE) presents an interesting problem in environmental risk assessment. Acutely, it is much less toxic than chlorinated hydrocarbon solvents that were developed early (e.g., carbon tetrachloride, chloroform) (*1*). The chlorinated hydrocarbons had replaced the extremely flammable petroleum distillates used previously for many industrial applications. Because of the combination of low acute toxicity and flammability, TCE came into broad use as a solvent and degreasing agent. This broad usage led to inappropriate disposal. The high mobility of TCE in the subsurface and the relative ease by which it can be measured at very low concentrations virtually guaranteed that it would become a pivotal chemical in the development and application of rules and regulations where intent has been to protect the public health (*2*).

The major economic reason for reexamining the risk assessment for TCE is that it is frequently a difficult problem for compliance with certain environmental laws, particularly the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Compliance with these laws has been responsible for much of the costs associated with cleanup of uncontrolled hazardous waste sites. The national costs for cleanup are huge, running into the billions of dollars (*3*). Therefore, relaxation of cleanup standards could save considerable public and private resources. However, reevaluation of this potential carcinogenic risk against proposed cancer risk assessment guidelines of the U.S. Environmental Protection Agency (U.S. EPA) (*4*) has broader implications. The metabolites of TCE thought responsible for its induction of liver tumors are also produced in the chlorination of drinking water (*5*). In the case of drinking water disinfection, the putative cancer risks from the formation of these compounds need to be balanced with the clearly established benefits of chlorination in the protection of public health from waterborne infectious disease (*5,6*).

Aside from its importance in the public policy arena, TCE is a classic example of a chemical that affects different target organs in different species and strains of experimental animals. This presents a challenge to risk assessment because the risk to humans can only be established by careful consideration of the mechanisms by which each cancer is produced. In some species, differing target organs may be attributed to quantitative differences in predominant pathways of metabolism (*7–9*). Clearly, different metabolites of TCE target different organ systems. However, there also appear to be some intrinsic species differences in susceptibility to the effects of these metabolites.

In general, the tumor site in each species or strain in which TCE induces cancer corresponds to those sites in which the spontaneous frequency is greater for that strain or species. It may be reasonably assumed that sufficient numbers of initiated cells are present in such organs and a variety of mechanisms could accelerate the development of these spontaneous tumors. The contribution of some of these mechanisms or modes of action to carcinogenesis may not be linear at low doses.

The focus of this review is the induction of hepatic tumors by TCE. The probability (possibility) that this response is strain specific will be considered in the context of which metabolites are most likely to be responsible. Second, the possibility must be considered that the same mechanisms that have been identified in the sensitive strain ($B6C3F_1$ mice) provide a more general warning or alert that TCE could present a hazard in humans for cancer. The effects of TCE on other target organs are considered by other authors (*8,9*).

In the course of this review, four modes of action are considered as they relate to the induction of liver tumors: somatic mutation, modification of cell signal pathways (peroxisome proliferation; other mechanisms that modify cell replication and death rates), cell death and reparative hyperplasia, and hepatomegaly/cytomegaly.

Ability of TCE to Induce Hepatic Cancer

TCE and several of its metabolites have been shown to produce hepatic cancer in experimental animals. Table 1 lists carcinogenesis bioassays in which TCE induces hepatic cancer, followed by a listing of studies in which liver tumor induction was evaluated but not found. Essentially, these data show that TCE only produces liver tumors in selected strains of mice ($B6C3F_1$ and Swiss) (*10–12*) despite adequate studies in several strains of rats (*13*) and in hamsters (*14*). A number of studies in other strains of mice have also been negative for liver tumor induction even though the dosing schedule and duration of the studies should have been

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Table 1. Evidence of liver tumor induction by trichloroethylene in experimental animals.

Species/strain/sex	Route/ method	Dose	Duration of treatment	Duration of observation	Liver tumor incidence (%)	Reference
Mice/B6C3F$_1$/M, F	Oral, COG	F: 869, 1,739 mg/kg M: 1,169, 2,339 mg/kg	78 weeks	90 weeks	F: 0, 5%; L, 52%; H, 65% M: 0, 0%; L, 8%; H, 23%	(*10*)
Rats/Osborne-Mendel/M, F	Oral, COG	B: 549, 1,097 mg/kg	78 weeks	110 weeks	0 incidence at all doses	(*10*)
Mice/NMRI/M, F	Inhalation	B: 0, 100, 500 ppm	18 months	30 months	M: 0, 6.6%; 100, 6.9%; 500, 0% F: 0, 0%; 100, 0%; 500, 0%	(*14*)
Rats/Wistar/M, F	Inhalation	B: 0, 100, 500 ppm	18 months	36 months	M: 0, 0%; 100, 3.3%; 500, 0% F: 0, 0%; 100, 3.3%; 500, 0%	(*14*)
Hamsters/Syrian/M, F	Inhalation	B: 0, 100, 500 ppm	18 months	30 months	M: 0, 0%; 100, 0%; 500, 0% F: 0, 3.4%; 100, 3.4%; 500, 0%	(*14*)
Mice/ICR/F	Inhalation	F: 50, 150, 450 ppm	104 weeks	107 weeks	0, 0%; 50, 0%; 150, 0%; 450, 2%	(*16*)
Rats/Sprague-Dawley/F	Inhalation	F: 50, 150, 450 ppm	104 weeks	107 weeks	0, 0%; 50, 2%; 150, 0%; 450, 2%	(*16*)
Mice/ICR-HA/M, F	Oral, COG	M: 2.4 g/kg F: 1.8 mg/kg	18 months	106 weeks	M: 0, 6%; 2.4, 10% F: 0, 0%; 1.8, 2%	(*15*)
Rats/ACI/M, F	Oral, COG	B: 500, 1,000 mg/kg	103 weeks	103 weeks	M: 0, 2%; L, 2%; H, 2% F: 0, 0%; L, 0%; H, 0%	(*13*)
Rats/August/M, F	Oral, COG	B: 500, 1,000 mg/kg	103 weeks	103 weeks	M: 0, 0%; L, 0%; H, 0% F: 0, 4%; L, 0%; H, 0%	(*13*)
Rats/Marshall/M, F	Oral, COG	B: 500, 1,000 mg/kg	103 weeks	103 weeks	M: 0, 2%; L, 0%; H, 0% F: 0, 0%; L, 0%; H, 0%	(*13*)
Rats/Osborne-Mendel/M, F	Oral, COG	B: 500, 1,000 mg/kg	103 weeks	103 weeks	M: 0, 0%; L, 0%; H, 2% F: 0, 0%; L, 0%; H, 0%	(*13*)
Mice/B6C3F$_1$/M, F	Oral, COG	B: 1,000 mg/kg	105 weeks	105 weeks	M: 0, 29%; 1,000, 78% F: 0, 13%; 1,000, 45%	(*11*)
Rats/F344/M, F	Oral, COG	B: 500, 1,000 mg/kg	103 weeks	103 weeks	Not significant	(*11*)
Rats/Sprague-Dawley/M, F	Inhalation	B: 100, 300, 600 ppm	104 weeks	Until death	Not significant	(*12*)
Mice/B6C3F$_1$/M, F	Inhalation	B: 100, 300, 600 ppm	78 weeks	Until death	M: 0, 3.3%; 100, 1.1%; 300, 3.3%; 600, 6.2%[a] F: 0, 2.2%; 100, 4.4%; 300, 4.4%; 600, 10%	(*12*)
Mice/B6C3F$_1$/M, F	Inhalation	M: 100, 300, 600 ppm	78 weeks	Until death	0, 18.9%; 100, 21.1%; 300, 30%; 600, 23.3%	(*12*)
Mice/Swiss/M, F	Inhalation	B: 100, 300, 600 ppm	78 weeks	Until death	M: 0, 4.4%; 100, 2.2%; 300, 3.3%; 600, 14.6% F: 0, 0%; 100, 0%; 300, 0%; 600, 1.1%	(*12*)

Abbreviations: B, both sexes; COG, corn oil gavage; F, female; H, high; L, low; M, male. [a]Early mortality resulted in second experiment in male B6C3F$_1$ mice.

adequate; e.g., ICR mice (*15*) and male and female ICR-HA mice (*14*). While TCE induced liver tumors only in mice administered by corn oil gavage, it produced tumors at other sites in mice via inhalation and rats via inhalation or gavage (*12,16*). It has also been associated with small excess risks for liver cancer and other tumor sites in epidemiologic studies (*17*).

The epidemiologic evidence provides some support for the hypothesis that TCE poses a liver cancer risk in humans; however, this evidence is not strong. The International Agency for Research on Cancer (IARC) (*1*) recently concluded that limited evidence existed for liver cancer but that risk was not consistent across all studies. IARC placed greatest weight on three studies of occupational exposure: the Spirtas et al. (*18*) study of aircraft workers and two studies of solvent-exposed workers, Axelson et al. (*19*) and Anttila et al. (*20*). When the results of all three studies were combined, the data appear to indicate elevated risks for liver and biliary tract cancer (relative risk [RR] = 1.79, 95% confidence interval [CI]: 1.13–2.68). RR was not elevated to a statistically significant degree in any individual study. Weiss (*17*) also examined a slightly different database than IARC (*1*) and drew similar conclusions. Chief among the studies considered by Weiss were the studies of Spirtas et al. (*18*), Axelson et al. (*19*), Anttila et al. (*20*), and one other study of the Hughes Aircraft employees (*21*). These studies indicated a total of 16 cases of primary liver cancer (a category that excludes cancer of the biliary tract) in which 9.5 were expected. Weiss (*17*) cautioned that the modest size of the increased risk and the lack of an exposure trend in the Spirtas et al. (*18*) study provide limited support for a causal hypothesis.

Evidence of Somatic Mutation Produced by TCE

There have been several reviews published on the mutagenic effects of TCE and its metabolites (*22,23*). An update of the literature is included as a separate effort in this review (*24*). Briefly, TCE has demonstrated a very limited ability to induce point mutations or clastogenic effects in a variety of bacterial, yeast, or mammalian cells used for *in vitro* testing. Although there have been scattered positive results, most of these results are equivocal or have not been independently confirmed (*23*). Therefore, data on the mutagenic effects will be confined to considerations of evidence that a chemically induced somatic mutation plays a role in the development of liver tumors.

The only evaluations of genotoxicity data of TCE that are directly relevant to liver tumor induction were studies of *in vivo* unscheduled DNA synthesis in rats and mice (*25–27*). All three studies were negative. Less direct assessments of the interaction of TCE with DNA have been conducted, but the actual mechanisms involved have not been established. For example, Walles (*28*) and Nelson and Bull (*29*) reported that TCE was capable of inducing single-strand breaks (SSBs) in hepatic DNA of both rats and mice *in vivo*. SSBs can reflect a number of physiological responses as well as direct effects of a chemical on DNA.

Covalent binding of radioactivity from ^{14}C-TCE to DNA *in vivo* is very low

(*30,31*). No specific adducts could be isolated (*31,32*). Bergman (*32*) assumed that the radioactivity associated with DNA is largely accounted for by metabolic incorporation of C_1 fragments. A large fraction (65–90%) of the ^{14}C-TCE reported to bind to protein is actually metabolically incorporated as glycine and serine presumably via the intermediate glyoxylate (*33,34*). Once in this pool, the label can be incorporated into other amino acids, such as alanine, that were not measured. Therefore, simple association of label cannot be accepted as evidence of covalent interaction with TCE and any metabolites of its oxidative pathway (*34*). Glycine is also a precursor for DNA synthesis. Caution must still be exercised, as new studies that focus on incorporation of label using more sensitive techniques have been performed without apparent appreciation of this problem (*35*). While these studies certainly overestimated the amount of covalent binding of TCE to protein, additional work has provided evidence of covalent binding to protein by correction for metabolic incorporation (*34*) or through the use of immunochemical techniques (*36*). These data suggest that the ability of TCE or its oxidative metabolites to interact with hepatic DNA directly is very limited.

Elevated levels of products of lipid peroxidation and 8-hydroxy-2-deoxyguanosine/2-deoxyguanosine ratios have been observed in the liver of rats treated with TCE at a dose of 1,000 mg/kg but not at 500 mg/kg (*37*). Free radicals have also been trapped when TCE is incubated with liver slices *in vitro* (*38*). Consequently, there is the possibility that radicals may contribute to the carcinogenic response. In part, these effects could contribute to the tumor response by increasing mutation rates in the target tissues. However, these effects become less of a concern at lower doses because of the much higher levels of similar damage produced by the normal energy metabolism of the body. Oxidative stress is also closely associated with tumor promotion mechanisms (*39*). The ability of hepatocarcinogenesis metabolites of TCE to contribute to such responses is discussed in later sections of this review dealing with the effects of its metabolites.

A report by Anna et al. (*40*) indicated that TCE-induced tumors possessed a different mutation spectra in codon 61 of the H-*ras* oncogene than those observed in spontaneous tumors of control mice. Results of this type have been interpreted as suggesting that a chemical is acting by a mutagenic mechanism (*41*). As indicated in the review of Maronpot et al. (*42*), it is not possible to a priori rule out a role for selection in this process. However, differences in mutation frequency and spectra in this gene provide some insight into the relative contribution of different metabolites to TCE-induced liver cancer.

Anna et al. (*40*) and Ferreira-Gonzalez et al. (*43*) independently assessed the frequency of H-*ras* mutations and spectra in dichloroacetate (DCA)-induced tumors. These published data and those of historical controls for male B6C3F$_1$ mice (*40*) specifically at codon-61 of H-*ras* are displayed in Table 2. The mutation frequency in DCA-induced tumors does not differ significantly from that observed in spontaneous tumors. However, there is an obvious change in the mutation spectra in codon 61, involving a significant increase in the H-*ras*-61^{CTA} mutation at the expense of the H-*ras*-61^{AAA} mutation. The mutation spectra found in DCA-induced tumors has a striking similarity to those observed in TCE-induced tumors and is different than those of TCA-induced liver tumors.

New data are becoming available that have only been reported in abstract form (*44*). As noted in Table 2, the mutation spectra of mouse liver tumors obtained from mice treated with TCA appear to be different than those induced with DCA (*43*). Only 11 TCA-induced tumors, of which 5 had detectable mutations in codon 61 of H-*ras*, were utilized in this analysis. The new data (*44*) indicate a mutation frequency in a larger number of TCA-induced tumors (n = 30) of 0.53. The ratio of AAA:CGA:CTA in codon 61 was found to be 3:2:1 (*44*). DCA-induced tumors were found to have a low mutation frequency (0.33) and to have an excess of the CTA mutation (ratio = 6:7:7), showing the same shift from the AAA to the CTA mutation reported previously (*42*). In contrast with previous data, tumors were examined in animals that had been treated with TCE in an aqueous vehicle. These tumors had a lower mutation frequency than the previous data from tumors induced by TCE in a corn oil vehicle (0.24 vs 0.51). However, the mutations spectra were 3:2:5, similar to those reported previously (*42*) for TCE-induced tumors (ratio = 6:5:9). The lower mutation frequency and the relative enrichment of the CTA mutation with TCE and DCA suggest a common mechanism. Thus, DCA may play a role in the hepatocarcinogenicity of TCE in mice despite its low level of formation (discussed later). It is notable that a mutation frequency lower than historical controls, as seen with TCE when administered in water rather than corn oil and with DCA, are more consistent with the activity of other chemicals that act as tumor promoters (*42*). The relatively high mutation frequency found with TCA is atypical of other peroxisome proliferators (*42*).

Difficulties in interpreting mutation spectra are just coming to light. The presence or absence of *ras* mutations cannot be correlated with the extent to which *ras*-dependent pathways are expressed in the liver (*45*). Recently, it was found that tumors induced by DCA in female B6C3F$_1$ mice had a very low frequency of H-*ras* mutations (*46*). Consequently, it is difficult to conclude that the induction of H-*ras* mutations plays an initiating role for liver cancer in mice.

It is important to consider the large differences in blood levels of DCA that are achieved by minimally carcinogenic doses and those used in the experiments in which mutation spectra were measured. As indicated above, the concentrations of DCA measured in the blood of male B6C3F$_1$ mice drinking 0.5 g/L were found to be 2–3 μM, whereas in mice consuming 2 g/L of DCA concentrations were found to be 300 μM during the period of active water consumption (*47*). Blood concentrations of DCA in mice gavaged with 1,000 mg/kg TCE are less than 2 μM, the limit of quantitation in the early studies

Table 2. Mutation frequency and spectra with codon-61 of H-*ras* of hepatocellular adenomas and carcinomas from B6C3F$_1$ mice treated with TCE and its metabolites.[a]

Chemical (reference)	Number of H-*ras* 61/tumors	Mutation frequency	gln CAA	lys AAA	arg CGA	leu CTA
			Male mice			
Spontaneous (*42*)	183/333	0.55	150 (0.45)	106 (0.32)	50 (0.15)	21 (0.06)
Dichloroacetate						
1 g/L × 104 weeks (*43*)	6/13	0.46	7 (0.54)	1 (0.08)	3 (0.23)	2 (0.15)
3.5 g/L × 104 weeks (*43*)	16/33	0.48	17 (0.52)	3 (0.09)	8 (0.24)	5 (0.15)
5 g/L × 76 weeks (42)	40/64	0.63	24 (0.37)	11 (0.17)	14 (0.22)	15 (0.23)
Trichloroacetate (*43*)						
4.5 g/L × 104 weeks	5/11	0.45	6 (0.55)	4 (0.36)	1 (0.09)	0 (0)
Trichloroethylene (*42*)	39/76	0.51	37 (0.49)	12 (0.16)	10 (0.13)	17 (0.22)
			Female mice			
Spontaneous (*42*)	33/49	0.67	16 (0.33)	17 (0.35)	12 (0.27)	4 (0.08)
Dichloroacetate (*46*)						
3.5 g/L × 104 weeks	1/22	0.05	21 (0.95)	0 (0)	0 (0)	1 (0.05)

[a]Mutations at other codons were not included, although these tumors are kept as part of the denominator.

(*48,49*). The concentrations used to obtain mutation spectra published to date were taken largely from animals treated with 3.5 or 5 g DCA/L. Therefore, the effects of lower doses of DCA (0.1–0.5 g/L) are more appropriately considered for comparisons to TCE.

Modification of Cell-Signaling Pathways

Peroxisome proliferation. Elcombe (*50*) examined the relative dose–response relationships in Swiss mice and Wistar-derived rats to TCE. TCE significantly increased cyanide-insensitive acyl coenzyme A (acyl-CoA) activity in the mice at doses of TCE as low as 100 mg/kg per day for 10 days. The response approached a maximum as doses approached 500 mg/kg. Although this is not the mouse strain in which liver tumors have been induced by TCE, at least these data show consistent species congruence between peroxisome proliferation and liver tumorigenesis. TCE also induced peroxisome proliferation in Wistar rats; however; liver tumor induction has not been identified despite studies in several strains of rat (*10,11,13*).

The importance of chemicals that are peroxisome proliferators as carcinogenic hazards to humans is controversial. There are data which indicate that chemicals that induce peroxisome synthesis in rodent liver *in vivo* and isolated rodent hepatocytes *in vitro* fail to produce this response or are much less potent in inducing such responses in human hepatocytes (*51*). The reason for this species difference remains to be established. Despite a large amount of data that correlate peroxisome proliferation with carcinogenesis, the actual mechanism by which such chemicals actually produce cancer may be only loosely associated with peroxisome proliferation, per se. The most frequently cited basis is a lower level of expression of the peroxisome proliferator activated receptor alpha (PPARα) (*52*). The quantitative relationships between potency of these chemicals as rodent hepatocarcinogens and as peroxisome proliferators are frequently quite divergent.

Issemann and Green (*53*) isolated the PPARα from mouse liver that activated a response element to increase the activity of a reporter gene. This receptor responded to TCA at concentrations in the mM range, approximately the same range that would be reached in blood of mice by treatment with TCE at the doses (*54*) employed in the NCI and NTP bioassays. Recent experiments with mice with a targeted disruption of the PPARα gene abolished the pleiotropic responses of peroxisome proliferators. Neither hepatomegaly, peroxisome proliferation, or transcription activity of the genes that produce peroxisomal enzymes were observed in response to clofibrate or Wy-14,643 (*55*). Also, cytochrome P450s of the CYP4A family were not induced. More important, mice with the targeted disruption of the PPARα gene were insensitive to the carcinogenic activity of a potent peroxisome proliferator, Wy-14,643 (*56*). Mice of the same strain that had an intact PPARα gene did develop tumors. Thus, it is clear that many of the responses traditionally associated with peroxisome proliferators are mediated through the PPARα. However, such studies have not been conducted with TCE or any of its hepatocarcinogenic metabolites.

Despite the lack of response or reduced sensitivity of human cells to the pleiotropic responses to peroxisome proliferators, they do possess an analogous protein (*57*). Additionally, *cis*-acting peroxisome proliferator-responsive elements have been identified in the 5′-flanking region of the peroxisomal fatty acyl-CoA oxidase gene in humans (*58*). Therefore, a definitive explanation for this differential sensitivity has yet to emerge. It does appear that the activation of the receptor is frequently indirect. In some cases it appears that it is activated by an increase in the intracellular concentrations of fatty acids (*59*). Another possibility lies in the fact that the activity of PPARα is modified by formation of dimers with other forms of the receptor (*57*). This inhibitory protein has been referred to as hNUC1 or PPARγ. The lower levels of PPARα in human liver relative to the rodent liver may change the influence of the two receptors.

The PPARs heterodimerize with the 9-*cis*-retinoic acid receptor (RXR). The heterodimer is responsible for activating transcription. The RXR/PPARα heterodimer activates the PPAR response element (*60*), but the PPARα downregulates the transcriptional activity because of the thyroid hormone receptor, apparently by competing for RXR binding (*61*). In addition, the PPARα/RXR heterodimer can inhibit transactivation by the estrogen receptor (*62*). These interactions suggest that the PPARs may play an important role in the developmental biology of most species, including humans (*59*).

Cytotoxicity and reparative hyperplasia. Many chlorinated hydrocarbon solvents are cytotoxic to hepatocytes. In a number of cases there are convincing data to suggest that these effects contribute to the induction of liver cancer. Chloroform (*63*) and carbon tetrachloride (*64,65*) are perhaps the best examples.

TCE is much less cytotoxic to the liver than these other chlorinated solvents. Buben and O'Flaherty (*66*) found liver hypertrophy in Swiss-Cox mice at doses of TCE as low as 100 mg/kg for 6weeks, but there was little evidence of necrotic injury. There were some increases in serum glutamate–pyruvate transaminase levels but only at doses much greater than required for carcinogenesis (i.e., 2,400 and 3,200 mg/kg) and that were frequently fatal as a result of CNS depression. A necrotic response to TCE appears to require doses or dose rates significantly greater than those that induce liver cancer (*50,67–69*).

Robust studies of the dose–response relationships in TCE-induced modifications of cell replication rates do not seem to be available. Treatments with TCE ranging from single doses to repeated doses over several weeks were seen to increase rates of cell division in the liver of $B6C3F_1$ mice without attendant signs of necrosis (*50,68*).

Hepatomegaly/cytomegaly. Hepatomegaly is routinely observed in mice that are treated with hepatocarcinogenic doses of TCE. Buben and O'Flaherty (*66*) noted the effect with doses as low as 100 mg/kg per day for 6 weeks in Swiss-Cox mice. Tucker et al. (*69*) found significant increases in liver size when CD-1 mice were treated with 1, 2.5, or 5 g/L in drinking water for 6 months. These concentrations in drinking water would provide doses very similar to those used in the Buben and O'Flaherty (*66*) study using corn oil gavage. In $B6C3F_1$ mice, Dees and Travis (*68*) noted significant increases in the liver-to-body weight ratio within 10 days at 100 mg/kg and significant increases in absolute liver weights at 250 mg/kg. Therefore, hepatomegaly is observed at doses well below those shown to induce hepatic tumors in $B6C3F_1$ mice (Table 1).

Hepatomegaly can arise from increases in cell number or cell size. In the case of TCE, the effect is associated with significant decreases in the concentrations of DNA (expressed as mg/g wet weight) (*50,66*). This indicates that at least some of the effect is attributable to cytomegaly and cannot be entirely attributed to increased cell numbers.

Metabolism of TCE

The metabolism of TCE is schematically depicted in Figure 1. This is simply to identify where those metabolites considered most likely to produce hepatic tumors fit into the metabolic scheme. Other reviews will more thoroughly address the characteristics of the individual metabolic steps and their variation between species (*11*) and the pharmacokinetics of TCE and critical metabolites (*70*).

From the perspective of liver tumor induction, the oxidative pathway of TCE metabolism appears to be the most important. Three products of this pathway have been shown capable of inducing liver cancer in $B6C3F_1$ mice, chloral hydrate (CH) (*71*), trichloroacetate (TCA) (*72–74*) and DCA (*71–76*). As there are also some potential reactive intermediates in both the oxidative and glutathione conjugation pathways, much of this review will dwell on the question of

whether the hepatocarcinogenic effects of TCE can be accounted for by one or more of these three metabolites. If not, perhaps other metabolites may need to be invoked.

CH arises as a key intermediate in the oxidative pathway (*77,78*), as it is produced by rearrangement of a trichloroethylene–oxygen–cytochrome P450 complex (*79*). CH is either rapidly reduced to trichloroethanol (TCOH) or oxidized to TCA. In most species, TCOH is rapidly glucuronidated and the glucuronide is secreted into the bile. It is then secreted into the small intestine, hydrolyzed back to TCOH in the gut, reabsorbed and available to either be oxidized to TCA through CH as an intermediate, or reconjugated with glucuronic acid (*80–83*). Normal humans are proficient at glucuronidating TCOH relative to rodents, particularly mice (*9,82*). Deficiencies in the ability to glucuronidate chemicals are, however, relatively frequent in the human population.

The glutathione pathway of TCE does lead to the formation of a clearly mutagenic metabolite, dichlorovinylcysteine (*84*). The *N*-acetyl derivative of this metabolite has also been identified in both rat (*85*) and human urine (*86*), and it has been postulated responsible for renal tumor induction in the rat (*85*) and possibly in humans (*87–89*). The metabolites within this pathway have not been linked to liver tumor induction.

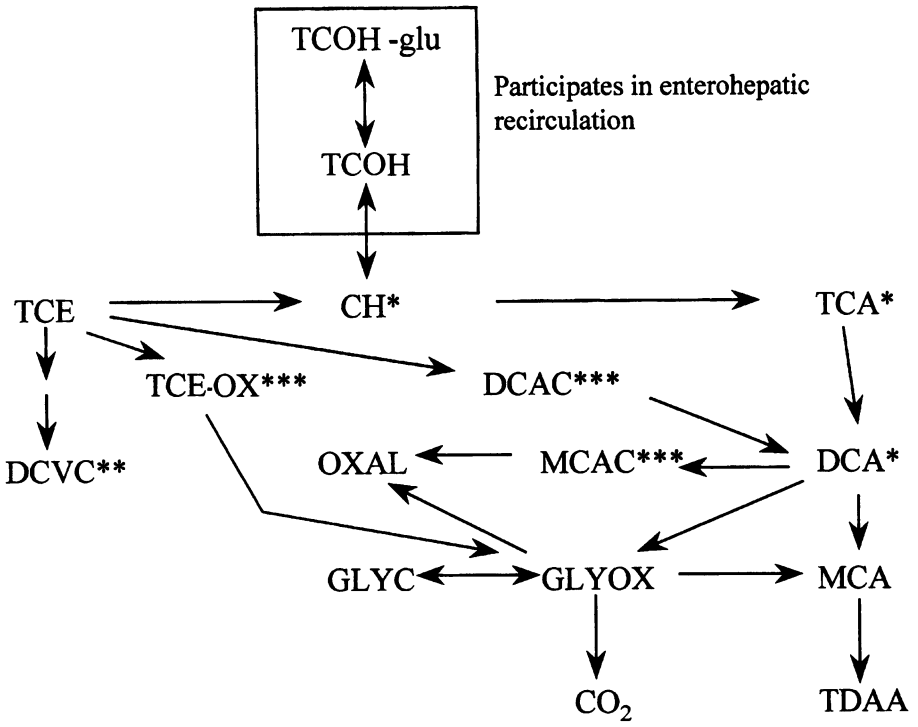

Figure 1. Simplified outline of trichloroethylene metabolism. *Hepatocarcinogenic metabolite; **metabolite proposed responsible for renal tumors; ***reactive intermediates. Abbreviations: CO_2, carbon dioxide; TDAA, thiodiglycolate; DCVC, dichlorovinylcysteine; GLYC, glycolate; GLYOX, glyoxylate; MCA, monochloroacetate; MCAC, monochloroacetyl chloride; OXAL, oxalate; TCOH-glu, trichloroethanol glucuronide; TDAA, thiodiglycolate; TCE-OX, trichloroethylene oxide.

Ability of Metabolites of TCE to Induce Hepatic Cancer

Table 3 lists studies that have associated hepatic tumor induction with various metabolites of TCE. These include trichloroacetaldehyde (CH), TCA, and DCA. It is notable that of these three metabolites, only DCA has been shown capable of inducing hepatic tumors in species other than the $B6C3F_1$ mouse. DCA induces hepatic tumors in both male and female $B6C3F_1$ mice (*72–75*) and male F344 rats (*90,91*). CH and TCA have been evaluated in F344 rats under similar conditions and did not induce hepatic tumors at similar doses that were employed for studies in mice (*92,93*).

The remainder of this review explores what is known of the mechanisms and modes of action of TCE and its metabolites to identify those variables that are important to the liver tumor response. Such an analysis is necessary to determine whether TCE poses a significant risk for liver cancer to humans exposed to environmental levels.

Hepatocarcinogenicity of Chloral Hydrate

Chloral hydrate has been reported to produce hepatic tumors in male $B6C3F_1$ mice in two studies. One study administered a single 10 mg/kg dose of CH by intragastric intubation to mice at 15 days of age (*94*). This led to statistically elevated levels of tumors between 48 and 92 weeks, but the results are based on the appearance of three adenomas and three carcinomas among eight animals (Table 3). In a second study, Daniel et al. (*71*) administered CH to male $B6C3F_1$ mice for 104 weeks in drinking water at an average dose of 166 mg/kg/day resulting in a 71% incidence of hepatic tumors (combined adenomas and carcinomas). The question of whether CH, itself, contributes directly to the carcinogenic effects of TCE is difficult to determine. Two of its metabolites, TCA and DCA, are sufficiently potent as hepatic carcinogens in $B6C3F_1$ mice to account for the response to CH.

Effects on the liver that could contribute to tumorigenesis. **Genotoxic effects.** IARC (*1*) concluded that CH was capable of clastogenic activity and of inducing aneuploidy. CH tends to be positive as a mutagen in *Salmonella typhimurium* TA100 but not in TA98 (*95*) or TA1535 (*96*). The activity toward TA100 was very weak in one assay (*95*) and substantially greater in another (*96*). It was notable that Waskell (*95*) recrystallized CH from alcohol 6 times before subjecting it to test, suggesting the lower activity was associated with a higher purity chemical. Bignami et al. (*96*) also performed other tests that indicate that CH was capable of inducing point mutations. A third group found CH to be negative in TA98, TA100, TA1535, TA1537, and TA1538 (*97*), and the purity of the CH was specified. Keller and Heck (*98*) could find no evidence of DNA–protein cross-links with CH treatment of isolated rat liver nuclei. A number of laboratories have shown that CH is capable of producing chromosomal aberrations *in vitro* (*22,99–102*), including aneuploid cells. Thus, chromosomal effects appear to be more consistently observed.

Testing of clastogenic activity of CH *in vivo* has produced inconsistent results. Russo et al. (*103*) demonstrated nondisjunction in mouse spermatocytes with intraperitoneal (i.p.) injections ranging from 80 to 400 mg/kg. On the other hand, Leuschner and Leuschner (*97*) were not able to detect increased frequency of micronuclei in bone marrow of mice or chromosome aberrations in rat bone marrow following CH treatment.

Cytotoxic effects and reparative hyperplasia. Hepatocellular necrosis was also observed in 2 of 10 male rats treated with concentrations of either 1,200 or 2,400 mg chloral/L of drinking water administered for 90 days by Daniel et al. (*104*). The necrosis observed at the high dose was more severe than that observed at the low dose, providing some indication of a dose response. Neither dose produced any sign of hepatomegaly in the rat. If it is assumed that rats drink water to 10% of their body weight in a day, the

Table 3. Evidence of hepatocarcinogenic effects of metabolites of trichloroethylene in experimental animals.

Species (sex)	Dose	Duration (weeks)	Tumor site	Combined hyperplastic nodule and hepatocellular adenoma: Incidence	Combined hyperplastic nodule and hepatocellular adenoma: Tumor/*n* (multiplicity)	Hepatocellular carcinoma: Incidence	Hepatocellular carcinoma: Tumor/*n* (multiplicity)	Reference
				Trichloroacetate				
Mice								
$B6C3F_1$ (M)	0	61	Liver	2/22	0.09	0/22	0	(*72*)
	5 g/L	61	Liver	8/22	0.5	7/22	0.5	
$B6C3F_1$ (M)	0	52	Liver	1/35	0.03	0/35	0	(*73*)
	1 g/L	52	Liver	5/11	0.45	2/11	0.18	
	2 g/L	52	Liver	15/24	1.04	4/24	0.17	
	2 g/L	37	Liver	2/11	0.18	3/11	0.27	
$B6C3F_1$ (M)	0	60–95	Liver	NR	NR	6.7–10%	0.07–0.15	(*75*)
	0.05 g/L	60	Liver	NR	NR	22%	0.31	
	0.5 g/L	60	Liver	NR	NR	38%	0.55	
	4.5 g/L	95	Liver	NR	NR	87%	2.2	
	5 g/L	60	Liver	NR	NR	55%	0.97	
$B6C3F_1$ (F)	0	52	Liver	1/40	0.03	0/40	0	(*74*)
	0.35	52	Liver	6/40	0.15	0/40	0	
	1.2	52	Liver	3/19	0.16	0/19	0	
	3.5	52	Liver	2/20	0.10	5/20	0.25	
	0	81	Liver	2/90	0.02	2/90	0.02	
	0.35	81	Liver	14/53	0.	0/53	0	
	1.2	81	Liver	12/27	0.	5/27	0.	
	3.5	81	Liver	18/18	1.0	5/18	0.28	
Rats								
F344 (M)	0	104	Liver	2/23	0.087	0/23	0	(*92*)
	0.05 g/L	104	Liver	2/24	0.083	0/24	0	
	0.5 g/L	104	Liver	5/20	0.25	0/20	0	
	5 g/L	104	Liver	1/22	0.045	1/22	0.045	
				Dichloroacetate				
Mice								
$B6C3F_1$ (M)	0	61						(*72*)
	5 g/L	61	Liver	25/26	4.6	21/26	1.7	
$B6C3F_1$ (M)	1 g/L	52	Liver	2/11	0.3	NR	NR	(*73*)
	2 g/L	52	Liver	23/24	3.6	5/24	0.25	
	2 g/L	37	Liver	7/11	2.2	0/11	0	
$B6C3F_1$ (M)	0	60	Liver	0/10	0	0/10	0	
	0.5 g/L	60	Liver					(*76*)
	3.5 g/L	60	Liver	12/12	2.3	8/12	1.7	
	5 g/L	60	Liver	27/30	2.3	25/30	2.2	
	0	75	Liver	2/28	0.07			
	0.05 g/L	75	Liver	4/29	0.31			
	0.5 g/L	75	Liver	3/27	0.11			
	0	104	Liver	1/20	0.05	2/20	0.1	(*71*)
	0.5 g/L	104	Liver	12/24	0.5	15/24	0.63	
$B6C3F_1$ (F)	0	52	Liver	1/40	0.03	0/40	0	(*74*)
	0.28	52	Liver	0/40	0	0/40	0	
	0.93	52	Liver	3/20	0.20	0/20	0	
	2.8	52	Liver	7/20	0.45	1/20	0.1	
	0	81	Liver	2/90	0.02	2/90	0.02	
	0.28	81	Liver	3/50	0.06	0/50	0	
	0.93	81	Liver	7/28	0.32	1/28	0.04	
	2.8	81	Liver	16/19	5.6	5/19	0.37	
$B6C3F_1$ (M)	0	100	Liver	14/50	0.25	5/50	0.28	(*76*)
	0.05	100	Liver	11/33	0.5	NR	NR	
	0.5	100	Liver	11/24	0.32	5/24	0.68	
	1	100	Liver	23/32	0.8	16/32	1.29	
	2	100	Liver	13/14	0.85	6/14	2.47	
	3.5	100	Liver	8/8	0.64	4/8	2.9	
Rats								
F344 (M)	0	60	Liver	0/7	0	0/7	0	(*91*)
	0.05 g/L	60	Liver	0/7	0	0/7	0	
	0.5 g/L	60	Liver	0/7	0	0/7	0	
	2.4 g/L	60	Liver	26/27	0.96	1/27	0.04	
	0	104	Liver	1/23	0.04	0/23	0	
	0.05	104	Liver	0/26	0	0/26	0	
	0.5	104	Liver	9/29	0.31	3/29	0.1	
	2.4	104	Liver	Not done	Not done	Not done	Not done	

(*continued*)

Table 3. *Continued.*

Species (sex)	Dose	Duration (weeks)	Tumor site	Combined hyperplastic nodule and hepatocellular adenoma: Incidence	Combined hyperplastic nodule and hepatocellular adenoma: Tumor/*n* (multiplicity)	Hepatocellular carcinoma: Incidence	Hepatocellular carcinoma: Tumor/*n* (multiplicity)	Reference
				Chloral hydrate				
Mice								
$C_{57}BLXC_3HF_1$	0	92	Liver	0/19	0	2/19	0.11	(*94*)
(single dose to	5 mg/kg			2/9	0.22	1/9	0.11	
neonatal mice)	10 mg/kg			3/8	0.38	3/8	0.38	
$B6C3F_1$	0	104	Liver	1/20	0.05	2/20	0.10	(*71*)
	1			8/24	0.33	11/24	0.46	

NR, not reported.

no-effect level of 1,200 mg/L corresponds to approximately 120 mg/kg per day. This would amount to about 18 g per day in an adult human, close to the estimated doses in the study by van Heijst et al. (*105*) discussed more fully below.

In contrast to findings in rats, both CD-1 and $B6C3F_1$ mice display hepatomegaly with high doses of CH. Doses of 144 mg/kg were administered by gavage for a period of 14 days (*106*). No effect was observed at 14.4 mg/kg. Longer term exposures of up to 104 weeks produced similar effects at 166 mg/kg (*71*). Parallel data on pathology and serum enzyme levels indicate that CH was minimally cytotoxic at these doses in mice. Therefore, the apparent greater sensitivity of mice (albeit of another strain) to the hepatocarcinogenic effects of CH than rats appears to parallel the sensitivity for cytomegaly. Clearly, the liver-enlarging effects of CH are observed in the same dose range (166 mg/kg) at which tumors were induced (*71*). Neither the effects nor the dose required to produce these effects appears consistent with a contribution of CH to liver tumor induction by TCE that is independent of the contributions of subsequent metabolites (DCA and TCA).

Hepatocarcinogenicity of Trichloroacetate

Trichloroacetate induces hepatocellular carcinomas when administered in drinking water to male and female $B6C3F_1$ mice (*72–74*). In these studies, dose-related increases in the incidence of malignant tumors and precancerous lesions have been obtained at concentrations in water of between 1 and 5 g/L with as little as 12 months of treatment (*73,74,92*). Significant increases in benign lesions were observed at concentrations as low as 0.35 g/L of drinking water. Under similar conditions of treatment, TCA has not induced hepatic tumors in F344 rats (*93*).

Effects on the liver that could contribute to tumorigenesis. **Genotoxic effects.** Early reports indicated that TCA induced SSBs in the hepatic DNA of mice and rats (*28,107*). Subsequent experiments using the same methodology (*108*) failed to replicate the increase in SSBs observed in mice, using a single, high 500 mg/kg dose. Using slightly different methodology, Chang et al. (*109*) found a very small but statistically significant increase in SSBs with a dose of 1,600 mg/kg TCA to mice but no effect at 800 mg/kg.

More recent data indicate that an oral dose of 300 mg/kg TCA has been shown to increase oxidative damage to DNA, as measured by increased content of 8-hydroxy-2-deoxyguanosine (8-OH-dG) content of nuclear DNA of the liver when administered in acute doses (*110*). However, this response was small and not observed when male $B6C3F_1$ mice were treated with 2 g TCA/L in drinking water for 3 or 10 weeks (*111*). Nevertheless, lipofuscin deposits are prominent in the liver of chronically treated mice, demonstrating some ongoing oxidative stress in response to TCA (*73*). Lipofuscin is, however, absent from TCA-induced tumors.

The totality of the data that are available suggests that TCA is unlikely to damage DNA directly, although there may be some damage that is induced by indirect mechanisms at very high doses. It is important to recognize that oxidative damage to DNA is rapidly repaired (*112*). Therefore, the small changes in the background levels of 8-OH-dG in DNA produced at very high doses may play little, if any, role in TCA's contribution to the hepatocarcinogenic responses to TCE.

Bull et al. (*73*) found that suspension of treatment at 37 weeks resulted in a smaller number of total tumors in mice at 52 weeks than in mice that had been treated continuously for the entire 52 weeks based upon total dose administered. These data suggest that benign lesions induced by TCA regressed when treatment was suspended at 37 weeks and support the hypothesis that TCA is not genotoxic. However, most of the tumors that remained in the group in which treatment was suspended were hepatocellular carcinomas (3 of 5). This was in contrast to the fact that only 4 of 16 tumors were found to be hepatocellular carcinomas in mice kept on continuous treatment.

The initiation–promotion study of Pereira and Phelps (*113*) also included a suspended treatment segment. These experiments were conducted in female rather than in male $B6C3F_1$ mice. TCA increased the yield of both hepatocellular adenomas and carcinomas in methylnitrosourea (MNU)-initiated mice (Figure 2). TCA significantly increased the yield of hepatocellular carcinoma as well as hepatocellular adenomas after 362 days of treatment. The mice whose treatments were suspended at 37 weeks had significantly reduced numbers and incidences of hepatocellular carcinomas at 52 weeks relative to initiated mice treated for the full 52 weeks with TCA. The hepatocellular carcinoma yield within the group that had its treatment suspended was very close to what would have been expected from adding the incidence of the MNU-only and TCA-only treatment groups. In the initiated mice, the incidence of hepatocellular adenomas was increased to essentially the same multiplicity whether at 31 weeks, 52 weeks, or in animals whose treatments were suspended at 31 weeks and who were sacrificed at 52 weeks. Hepatocellular adenomas were very few in mice treated with TCA alone. While hepatocellular adenomas

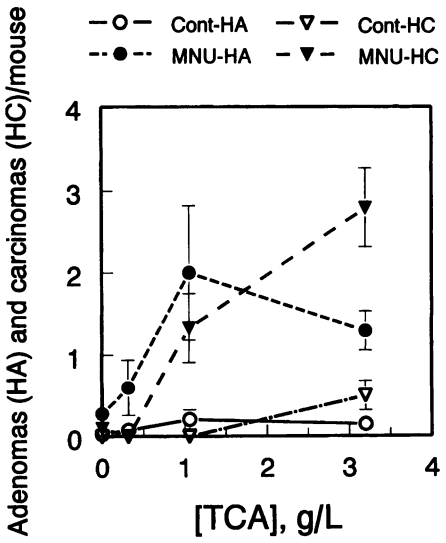

Figure 2. The ability of TCA to promote the formation of liver tumors in $B6C3F_1$ mice that have been previously exposed to MNU as an initiator. The vertical bars indicate SEM. Adapted from Periera and Phelps (*113*).

were at a higher incidence and multiplicity with TCA than with MNU alone, they appeared remarkably insensitive to any change in the treatment period for TCA. There is a consistency with the observations of Bull et al. (*73*) that suggests that the hepatocellular carcinomas arise from the similar cell populations with TCA treatment in both the whole carcinogenesis and the initiation–promotion study.

Another publication (*114*) reports that 27% of TCA-promoted tumors derived from the B6C3F$_1$ hybrid mice from the Pereira and Phelps (*113*) study lost heterozygosity on chromosome 6. There was no loss of heterozygosity in this chromosome in DCA-promoted tumors, indicating that the effect was not attributable to the initiator. As a consequence, these data imply that either the initiated cells promoted by TCA to hepatocellular carcinomas are susceptible to this clastogenic result spontaneously (something that is known to happen both clinically and experimentally) or that TCA could be acting as a tumor progressor. The very weak activity of TCA to act as a clastogen provides some basis for the former hypothesis (*115*).

Modification of cell-signaling pathways. TCA induces peroxisome proliferation in male B6C3F$_1$ mice over the same dose range that it induces hepatic tumors (*76*). Unlike the situation with DCA, discussed in the next section, increased expression of peroxisomes by TCA appears to be sustained over time. TCA also induces peroxisome synthesis in F344/N rats, but the increases in acyl-CoA oxidase activity were only 2–3 times the control levels at the concentrations administered in the drinking water (*93,116*). This compares to a 10-fold increase in this peroxisome marker with similar dosing schedule in mice. It is rare that such a small effect on peroxisome proliferation would be tied to carcinogenesis.

It is notable that Elcombe (*50*) found that the Wistar rat was actually more sensitive than mice to increases in cyanide-insensitive acyl-CoA oxidase activity induced by TCA. Obviously, this is inconsistent with the relative sensitivity of these two species to the tumorigenic effects of TCA in that rats (in this case F344) appear to be less sensitive than mice (B6C3F$_1$) (*116*). These results may reflect a strain difference or they may be attributed in part to the weak peroxisome proliferative activity of corn oil, which was the vehicle used by Elcombe (*50*).

Modifications in rates of hepatocyte replication and death. The available data suggest that TCA has tumor-promoting activity. Stauber and Bull (*117*) induced tumors with TCA at 2 g/L for 50 weeks. In Figure 3, the replication rates of TCA-induced tumors are shown. The rates within tumors from mice in which TCA administration had been suspended for 2 weeks still have a very high labeling index, matching those rates observed in tumors of mice with continuous treatment. Thus, there was no indication that the replication rates within tumors were modified by TCA treatment. In contrast, TCA treatment sharply depressed replication rates in normal hepatocytes in the same mice in which the long-term treatment was maintained until sacrifice. Thus, these results appear to be consistent with the negative selection hypothesis that has been advanced to explain the activity of other tumor promoters (*118*). This hypothesis suggests that cells resistant to downregulation of mitogenic effects of a chemical have a growth advantage over normal cells in an organ.

Recently, TCA has been shown to stimulate the growth of colonies of cells obtained from the liver of B6C3F$_1$ mice to grow in soft agar (*119*). These colonies express the c-*Jun* phenotype characteristic of TCA-induced tumors. This is in contrast to the c-*Jun*+ phenotype observed with DCA-induced liver tumors in mice (discussed more fully below). These data provide strong evidence that TCA is acting primarily by increasing the clonal expansion of a specific group of initiated cells within the liver of the B6C3F$_1$ mouse.

Hepatomegaly/cytomegaly. As with other peroxisome proliferators, treatment with TCA is associated with increases in liver weight. Increases in liver weight appeared to be more or less linear with dose with exposures as short as 14 days to as much as 90 days (*120,121*). These effects have been consistently observed at doses of TCA as low as 0.3 g/L or approximately 100 mg/kg per day in B6C3F$_1$ mice.

Hepatocarcinogenicity of Dichloroacetate

Dichloroacetate is an effective inducer of hepatic tumors in both mice and rats. Several studies in male and female B6C3F$_1$ mice found multiple tumors per animal with treatment concentrations of 2 g/L and above with as little as 40 weeks of treatment (*71–75,117*). These studies are summarized in Table 3. At 12 months of treatment the dose response is very steep, with essentially no response observed at concentrations of 1 g/L but as many as 4 tumors/liver in mice treated with 2 g/L (*73*). However, concentrations as low as 0.5 g/L will result in a hepatic tumor incidence of approximately 80% in a full 2-year study (*76*).

Hepatic tumors are also induced by DCA in male F344 rats (*90,91*). High doses of DCA given to rats produced a peripheral neuropathy (*122,123*) that complicated the conduct of cancer bioassays. Nevertheless, increased incidence of hyperplastic nodules and hepatocellular adenomas and carcinomas was observed at 60 weeks of treatment at 2.4 g/L (Table 3). As in mice, if DCA treatment was extended to 104 weeks, the incidence of these lesions was 41% in a group of 29 rats at a treatment concentration of 0.5 g/L. No tumors were observed at 0.05 g/L and only one hepatic tumor was observed in 33 control rats.

Effects on the liver that could contribute to tumorigenesis. **Genotoxic effects.** The ability of DCA to induce damage to DNA that

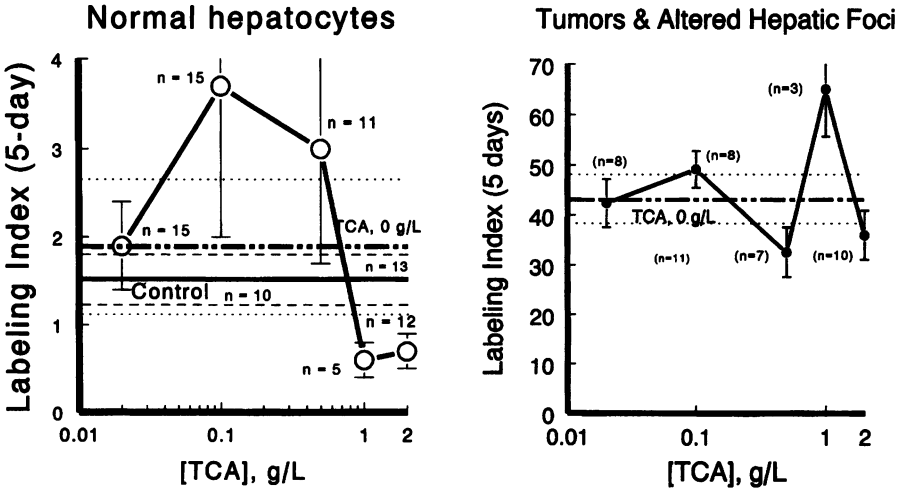

Figure 3. Effect of TCA on replication rates in normal hepatocytes and within tumors and altered hepatic foci. Male B6C3F$_1$ mice were treated with 2 g TCA/L of drinking water for 50 weeks. At that time they were transferred to drinking water containing the concentrations indicated on the *x*-axis for two additional weeks. Miniosmotic pumps were implanted to deliver BrdU for 5 days. Replication rates were measured by counting cells positive to an anti-BrdU antibody relative to total numbers of cells. Rates of replication in animals that were initially treated with TCA and transferred to distilled water for 2 weeks are denoted by TCA 0/g/L with dotted lines indicating the SEM. The solid line labeled control indicates replication rates in the liver of mice carried as controls throughout the whole experiment, with the SEM indicated by the dashed lines. Each point is labeled with the number of animals or lesions scored ± SEM. Data from Stauber and Bull (*117*).

could give rise to mutations or chromosomal damage has received study both *in vivo* and *in vitro*. Classical evaluations of DCA in *Salmonella* tester strains, both with and without metabolic activation, have been largely negative if held to the standard of at least a 2-fold increase in apparent mutation frequency to identify a compound positive (*95,124*). However, a number of more recent studies have suggested some potential for DCA-induced modifications in DNA.

***In vitro* or bacterial systems.** DeMarini et al. (*125*) reported that DCA induced prophage in *E. coli* at concentrations of 0.26 mM and produced 2.7 and 4.2 revertants/ppm in *Salmonella* TA100 with and without S9 addition, respectively. There are some difficulties in interpreting this report, as the author introduced DCA as a vapor and it is not clear whether the concentrations reported (i.e., ppm) refer to air or media concentrations. Also, at least in the case of the *Salmonella* assay, the DCA was introduced as the free acid and allowed to vaporize and partition into the incubation media. DCA is a strong acid with a pK_a = 1.3 (*126*). If DCA is adequately volatilized and sufficient time is allowed to come to equilibration, ion trapping would result in near quantitative transfer of DCA to the media, which in this case would be approximately 0.06–0.6 M. Brusick (*127*) has documented genotoxic effects that are induced by low pH. The amount of DCA introduced into the prophage assay is unclear because the method of addition was not described, although the introduction implied that it was again being tested as a volatile substance.

Giller et al. (*128*) examined a series of haloacetic acids, including DCA, in the SOS chromotest, the Ames' fluctuation assay, and the newt micronucleus assay. DCA was reported mutagenic in the Ames' fluctuation assay, at concentrations > 100 µg/mL in the absence of rat liver S9 fraction and > 1,000 µg/mL in the presence of S9. There was also a positive response with the SOS chromotest at 500 µg/mL. The newt micronucleus assay was negative. It appears that the free acid was also used in these studies, as there was no indication that the acids were neutralized. Therefore, the value of these data is limited because of the potential of pH artifacts (*127*).

The mutagenic activity of sodium DCA was explored in a variety of test systems by Fox et al. (*129*). These investigations found no evidence of increased mutation rates in *Salmonella* tester strains TA98, TA100, TA1535, or TA1537; *E. coli* strain WP2urvA; or the mouse lymphoma forward mutation assay whether incubated in the presence or absence of rat liver S9 fraction for metabolic activation. These authors found no evidence that DCA was capable of inducing chromosomal aberrations in Chinese hamster ovary cells *in vitro* at doses up to 1,100 mg/kg for 3 days. These studies used neutralized DCA, supporting the contention that positive results in prior studies may have resulted from artifacts secondary to the testing of the free acid or because different sources of DCA may have greater amounts of impurities.

More convincing evidence of mutagenic effects of DCA was obtained by Harrington-Brock et al. (*115*) in mouse lymphoma cells. DCA was found to increase the thymidylate synthetase mutant frequency in a concentration range of 100–800 µg/mL. Cytogenetic analyses documented an increased frequency of chromosomal aberrations at 600 and 800 µg/mL.

The concentrations at which these genotoxic effects occur are important in determining whether they play a role in either DCA- or TCE-induced liver cancer. The lowest dose of DCA that has been shown to produce an increased incidence of cancer is 0.5 g/L (*71*). A concentration of 0.05 g/L was found ineffective (*76*). The peak concentrations of DCA that are observed with the 0.5-g/L treatment are approximately 2–3 µM [approximately 25–40 pg/mL (*45*)]. Concentrations of DCA in the blood produced from the metabolism of high doses of TCE (1 g/kg) in mice fall below the limit of quantitation (*48,49*). Using these data and assuming the project down linearly with a physiologically based pharmacokinetic model, Barton et al. (*49*) estimated the area under the blood concentration time curve (AUC) of DCA obtained from 1,000 and 2,000 mg/kg TCE doses used in the cancer bioassays was 0.25 and 0.31 mg-hr/L. These values fall between the estimated AUCs in mice treated with 0.05 and 0.5 g/L in their drinking water (0.041 and 0.72 mg-hr/L, respectively). Available data suggest that less DCA is formed in the metabolism of TCE by rats and humans (*70*).

***In vivo* experiments.** DCA was reported to induce SSBs in hepatic DNA when administered by gavage to both mice and rats (*28,107*). Subsequent investigators were unable to repeat these results (*92,109*). A small transitory increase in SSBs was observed with doses of 5 and 10 µmol/kg in male $B6C3F_1$ mice by Chang et al. (*109*). More recently, Austin et al. (*110*) have shown that acute doses of DCA produce oxidative damage to nuclear DNA, measured as 8-OH-dG. The time course of this damage is more consistent with the development of SSBs reported by Chang et al. (*109*) and could represent the repair processes that involve strand scission. There are two important points that must be made: *a*) the induction of SSBs by Chang et al. (*109*) was very small relative to that seen with the positive controls, diethylnitrosamine and methylmethane sulfonate; and *b*) although increased 8-OH-dG was observed with acute treatments with DCA, there was not a sustained elevation of this adduct in nuclear DNA of mice when treatments with carcinogenic concentrations in drinking water were carried out for 3–10 weeks (*111*).

Fuscoe et al. (*130*) reported results obtained with the mouse peripheral blood erythrocyte micronucleus assay. They found a small but statistically significant increase in polychromatic erythrocytes containing micronuclei in male $B6C3F_1$ mice treated for 9 days with 3.5 g DCA/L of drinking water. This effect was not maintained through 28 days of exposure. These investigators also examined DNA migration in the single-cell gel assay. In this case, DCA appeared to retard migration of DNA, suggesting the possibility of DNA cross-linking (as opposed to SSBs) after 28 days of treatment at 3.5 g/L. Neither assay revealed significant effects of DCA at concentrations of DCA of 2 g/L or below. It is important to recognize that DCA induces 3 to 4 tumors per animal within 1 year at 2 g/L in drinking water (*73*). The higher dose adds little to the tumorigenic response. Moreover, the hepatic tumor incidence at 0.5 g/L is > 80% incidence, as observed in lifetime exposures to levels of DCA as low as 0.5 g/L (*76*). This would suggest that this evidence of potential genotoxic effects at high doses may have little to do with the induction of hepatic cancer by DCA.

Leavitt et al. (*131*) reported increased recovery of mutant cells from the *lacI* transgenic mouse with varying periods of treatment with DCA in drinking water. Significant increases were observed only when mice had been treated with 3.5 g/L for 60 weeks, not at shorter time intervals. No significant increases were noted at 1 g/L. While the authors took care to ensure that nodules and tumors were excluded from the sampling, Stauber and Bull (*117*) found that there are numerous lesions that were smaller than nodules in $B6C3F_1$ mice maintained on 2 g/L dichloroacetate for only 40 weeks. It was inevitable that some of these microscopic lesions were included within the tissue samples described. Given the marked stimulation of cell replication that occurs within lesions in mice, it is probable that the effect reported by Leavitt et al. (*131*) was due to the ability of DCA to selectively stimulate the growth of tumor precursor lesions.

Modification of cell-signaling pathways. Like TCA, DCA is a weak peroxisome proliferator in mice (*116*). However, this response is not stable, generally disappearing after a few weeks treatment. There is also a clear differentiation between the lowest dose that induces tumors, 0.5 g/L (*76*), and the lowest dose that produces sustained peroxisome proliferation, 3.5 g/L (*76*).

DCA has a considerable history as a hypoglycemic agent (*132*). In part, this is probably due to the established effects of DCA of inhibiting the pyruvate dehydrogenase kinase (*133*). More recent data have shown that administration of DCA substantially lowers serum insulin concentrations in mice treated with 0.5 or 2 g/L of DCA for 2 or more weeks (*134*) without significantly affecting plasma glucose concentrations (*47*). Serum insulin concentrations appear actually to be increased in mice treated with low i.p. doses of DCA (≥ 10 mg/kg) presented with a glucose challenge (*47*). Consequently, this effect is not due to inhibition of insulin secretion. The blood concentrations that are effective in reducing serum insulin concentrations are in the range of 1–2 μM. The apparent K_i of DCA for the pyruvate dehydrogenase kinase is 200 μM (*133*), a concentration that can be achieved in blood of mice with 2 g/L in the drinking water (*47*). Consequently, it is unlikely that these two effects are produced by the same mechanism.

DCA increases the deposition of glycogen in the liver. The dose response for glycogen deposition in the liver is in the same range that is required for inducing hepatocarcinogenesis (*47*). The accumulation of glycogen with DCA treatment takes a pathological character, becoming resistant to mobilization by fasting after 8 weeks of treatment. Increased hepatic glycogen is capable of inhibiting glycogen synthase, an effect that is observed in the liver of DCA-treated mice (*47*). However, DCA does not inhibit glycogen synthase when incubated with liver homogenates *in vitro*. The correlation of doses raises the possibility that the accumulation of glycogen and induction of tumors by DCA could be produced by modification of the same cell-signaling pathways.

Overexpression of the insulin receptor 1 substrate in human liver cells is known to be a suppressor of transforming growth factor β_1 (TGF-β_1)-induced apoptosis (*135*). Since the tumors express elevated amounts of the insulin receptor relative to the surrounding tissue (*134*), increased sensitivity to insulin could contribute to liver tumorigenesis by DCA. Insulin receptor expression in the normal portions of the liver is sharply suppressed by DCA, an effect that is reversed by the suspension of treatment. Presumably, this would mean that DCA-induced tumors may be more resistant to TGF-β_1–induced apoptosis. Since DCA-induced tumors are uniformly glycogen poor, the accumulation of glycogen in normal hepatocytes could contribute to the differential effects of DCA treatment on insulin receptor expression in normal hepatocytes and tumors. Thus, it is possible that greater sensitivity of tumor cells to insulin than normal cells could contribute to liver tumor induction.

Modifications in the rates of hepatocyte replication and death. DCA appeared to specifically stimulate outgrowth of hepatocellular adenomas rather than carcinomas. Pereira and Phelps (*113*) examined the effects of DCA as a promoter of MNU-initiated hepatic tumors in male B6C3F$_1$ mice. These data are provided in graphic form in Figure 4. At a concentration of 2.6 g/L of drinking water, DCA induced a large increase in the number of hepatocellular adenomas but had no significant effect on the induction of hepatocellular carcinomas.

The data of Pereira and Phelps (*113*) appear to be at least partially consistent with the stop experiments of Bull et al. (*73*). The latter authors found that suspending the treatment of male B6C3F$_1$ mice with DCA at 37 weeks appeared to arrest progression of liver tumors but resulted in a yield of hepatocellular adenomas and nodules that were proportional to the total dose of DCA administered. The initiation–promotion experiments of Pereira and Phelps (*113*) included a suspension of treatment with DCA at 31 weeks. This resulted in a substantial decrease in the numbers of MNU-initiated hepatocellular adenomas in female B6C3F$_1$ mice at 52 weeks, with little effect on hepatocellular carcinoma yields. Nevertheless, there is an inconsistency with prior data. Bull et al. (*73*) found that the number of DCA-induced lesions were proportional to the total dose administered to the animal whether treatment was terminated at 37 or 52 weeks. The total number of lesions seen in the Pereira and Phelps (*113*) study was significantly less than would be predicted. It is probable that the differences between the two studies are related to the use of female rather than male mice and/or a tumor initiator in the latter study.

It is notable, however, that the total lesion numbers (foci + adenomas + carcinomas) observed by Pereira and Phelps (*113*) were not different depending upon whether the MNU-initiated animals were treated with DCA and sacrificed at 31 vs 52 weeks. The essential difference was that more lesions fell into the adenoma class at the expense of the foci class of lesions at 52 weeks. This suggests that DCA treatment acted to increase the rate at which lesions develop rather than increasing the number of lesions. The analysis of Pereira and Phelps (*113*) did not include measures of lesion size, but it is suggested that progression without increases in number is simply a function of clonal size.

More recent studies of the effects of DCA on cell replication within normal hepatocytes and altered hepatic foci (AHF) and tumors (predominately adenomas) indicate that it does have selective effects. Stauber and Bull (*117*) found that DCA

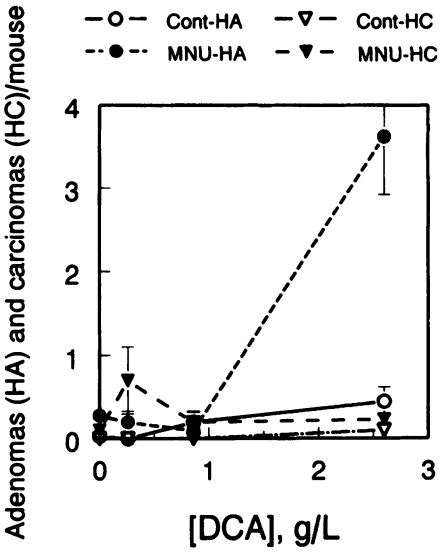

Figure 4. The ability of DCA to promote the formation of liver tumors in female B6C3F$_1$ mice that have been previously treated with a single dose of MNU as an initiator. The vertical bars indicate the SEM. Data adapted from Periera and Phelps (*113*).

had a small, stimulatory effect on the replication rate of normal hepatocytes over the first 14 days of treatment. As treatment was extended to 28 days and beyond, these effects became inhibitory at concentrations in drinking water of 0.5 g/L and above. In contrast, hepatocytes within nodules and tumors appeared to be resistant to the inhibitory effects of chronically administered DCA. At a concentration of 2 g/L, DCA doubled the rate at which cells that were immunoreactive to c-*Jun* and c-*Fos* antibodies replicated within hyperplastic nodules and adenomas (Figure 5). This strong stimulation of tumor cell replication appears to be responsible for the very rapid induction of tumors in mice treated with DCA in drinking water at concentrations of 2 g/L and above. It appears that the slower induction of liver tumors at lower doses of DCA depends primarily on the selective suppression of the replication of normal hepatocytes relative to initiated cells.

The work of Pereira (*121*) confirms the transient increase in the replication rates of hepatocytes observed with treatment with DCA. At 5 days of treatment there was a dose-related increase in the rate of replication at concentrations of 260–2,600 mg/L. No statistical analyses of these data were provided, but visual inspection of the mean values and estimates of their variability suggests that statistical significance might be approached at concentrations of 830 mg/L and above. This response was attenuated at 12 days of treatment and appears to be completely gone within 33 days of treatment.

Carter et al. (*136*) also observed the inhibitory effect of DCA on replication of normal hepatocytes. The rate of replication is

sharply inhibited within 5 days at DCA concentrations of 5 g/L. At 0.5 g/L the replication rate appeared to be inhibited to the same extent observed with 5 g/L after 20 days of treatment. These decreases in replication were accompanied by decreases in the percent of mononucleated cells, which Carter et al. indicate is probably associated with an increase in tetraploid cells. Therefore, while different experimental designs make it difficult to rectify the results, it appears that dosing levels of DCA of 2.6 g/L and below produce a transient increase in the replication rates of normal hepatocytes. Depressed rates of normal hepatocyte replication in DCA-treated mice relative to control animals as the treatment period becomes more prolonged appear to be a consistent observation across laboratory.

The suppression of cell replication in normal hepatocytes of mice by DCA-treatment is accompanied by decreased apoptosis in normal hepatocytes (*137*). At concentrations of 5 g/L the frequency at which apoptotic cells are observed drops 60–75% with as little as 5 days of treatment. At 0.5 g/L, there is a downward trend that is observed over the period from 5 to 30 days such that the frequency of apoptotic bodies at this low dose approaches that observed at the highest dose at 30 days. This result essentially parallels that described above for suppression of the rates of cell replication. This raises a question as to whether the driver of the response is suppressed replication or suppressed apoptosis. Whichever is the case, this translates into suppressed turnover of normal hepatocytes. The question is whether this suppressive effect on cell turnover increases the probability of transformation of hepatocytes or clonal expansion of damaged hepatocytes that would normally be eliminated.

Stauber et al. (*119*) determined the extent to which DCA could selectively stimulate the growth of clones of cells derived from the liver of naïve $B6C3F_1$ mice on soft agar. Figure 6 indicates the time-dose–response relationships that result when the only treatment of cells is the indicated concentrations within the Petri dish. DCA substantially increased the numbers of colonies over those that would grow out spontaneously. A most promising aspect of this result is that the colonies that grew out with DCA faithfully reproduced the phenotype (c-*Jun*+) of the tumors that were produced *in vivo*. The opposite phenotype was produced in TCA-induced tumors and in clones of cells derived from mouse liver that were grown in the presence of TCA on soft agar. A second experiment was conducted where male $B6C3F_1$ mice were treated with a minimally carcinogenic concentration of DCA in drinking water for 14 days and the *in vitro* segment of the experiment repeated. This experiment was predicated on the notion that if the main effect of DCA is to produce clonal expansion, then the number of cells isolated should increase with *in vivo* treatment. This occurred as predicted. However, it was also noted that the sensitivity of the cells obtained from treated animals to DCA in the soft agar had substantially increased. Figure 7 compares the dose–response curves for naïve animals and animals that had been pretreated. Statistically significant increases in the numbers of colonies were observed at the lowest concentration studied, 20 μM. There was no statistically significant effect on the number of colonies produced from plating a standard number of hepatocytes from naïve mice until the dose was increased above 200 μM.

Miller and co-workers (*138*) directly examined the effect of DCA treatment on liver tumor growth rates in mice *in vivo* using magnetic resonance imaging. DCA was administered to mice until small tumors became apparent (~1 mm in diameter). The animals with tumors were randomly assigned to two groups, one in which treatment was maintained and the other in which treatment was suspended, and the tumors imaged over the next 2–3 weeks. The results of this study are shown in Figure 8. Cessation of DCA treatment essentially abolished the growth of tumors. These data

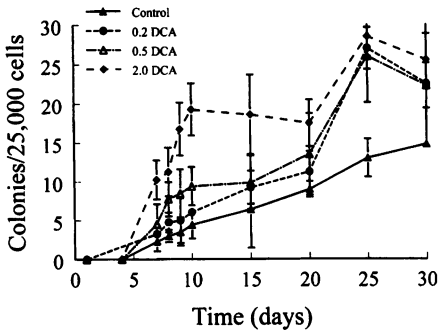

Figure 6. The time-course of colony formation when a hepatocyte suspension from naïve male $B6C3F_1$ mice is plated on soft agar with the indicated concentrations of DCA included in the agar. The increase in colony yield was significant with all three concentrations of DCA in the agar. Data from Stauber et al. (*119*).

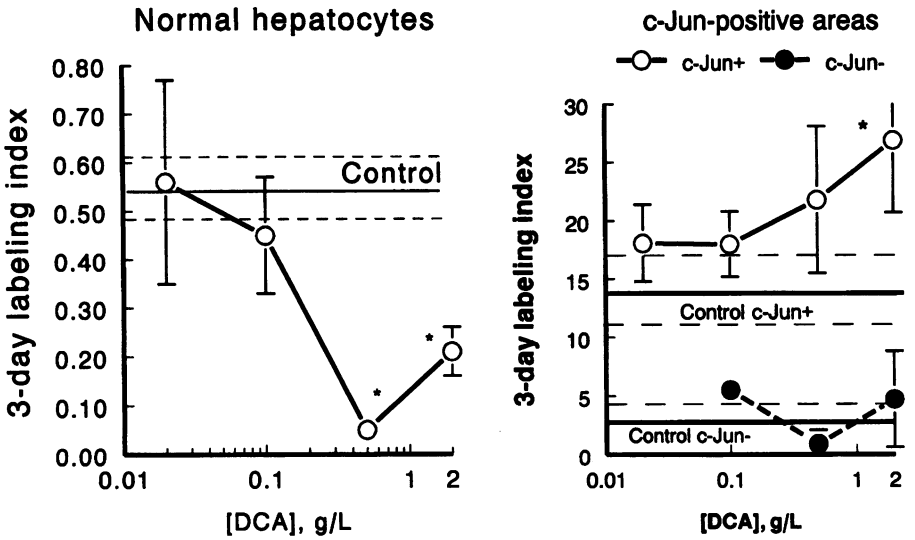

Figure 5. Effect of DCA on replication rates in normal hepatocytes and c-*Jun*+ and c-*Jun*- cells within altered hepatic foci and tumors. Experiment involved treatment of male $B6C3F_1$ mice for 38 weeks with 2 g DCA/L. At that time, animals were transferred to treatments coinciding with the concentrations identified on the *x*-axis for an additional two weeks. Cell replication rates were measured by measuring the number of cells that incorporated BrdU into their nuclei over a 3-day time interval. Replication rates in control animals, or in lesions in mice transferred to 0 g/L for 2 weeks prior to sacrifice are depicted by the solid lines, with the SEM marked by the dashed lines in both cases. Data from Stauber and Bull (*117*).

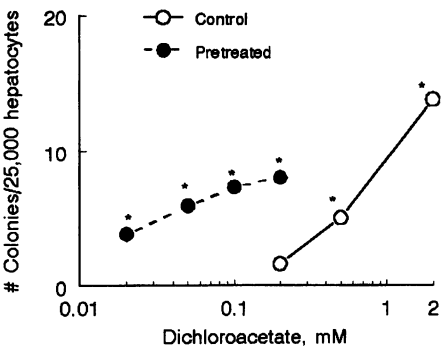

Figure 7. Effect of *in vivo* treatment of male $B6C3F_1$ mice with 0.5 g DCA/L of drinking water for 14 days on colony formation of hepatocyte suspensions plated on soft agar. Dose–response curves were constructed by subtracting out spontaneously growing colony counts from those obtained in the indicated concentration of DCA. Colony counts were made after 10 days in culture. Data points significantly different from colonies developed in control incubations are marked with an asterisk (*). Data from Stauber et al. (*119*).

were modeled to determine if the small lesion size distribution found in previous studies (*117*) could be accounted for by the effects of DCA on tumor growth. The growth data and prior measurements of the effects of DCA on replication rates within tumors (*117*), assuming a suppression of apoptosis in this cell population was of the same magnitude noted in normal hepatocytes of DCA-treated mice (*137*), accounted for the lesion size distribution.

Cytotoxicity and reparative hyperplasia. Early reports (*120*) indicated that high doses of DCA produced localized lesions in the liver. These lesions led to localized increases in the labeling index. Subsequent pathological analysis of slides identified the lesions as acinar necrosis (*139*). More recently, DeAngelo and co-workers (*76*) developed a dose–response characterization of the severity of these lesions. A curve derived from these data is provided in Figure 9. Necrosis never involved large portions of the liver as indicated by the relatively low scores, and was confined to treatment levels ≥ 1 g/L. No difference from control animals was observed at doses of 0.5 g/L. A liver tumor incidence of approximately 80% is observed at this treatment level (*76,117*). While these effects may contribute to the tumor response observed at high doses, they cannot be invoked as being responsible for the tumor responses seen at low doses.

Hepatomegaly/cytomegaly. Bull et al. (*73*) examined the effects of DCA on the liver of $B6C3F_1$ mice in some detail, with exposure lasting up to 1 year. As had been alluded to in earlier studies, DCA was found to produce a severe hepatomegaly in mice at doses at concentrations in drinking water of 1 g/L and above. The hepatomegaly could be mostly accounted for by large increases in cell size (cytomegaly). Hepatocytes from these mice stained very heavily for glycogen (PAS) despite the fact that the livers had been fixed in formalin. The accumulation of PAS began to occur with as little as 1–2 weeks of treatment (*120*) but became progressively more severe with time (*73,136*). These effects could not be replicated by exposing mice to the metabolites of DCA, glycolate, glyoxylate, or oxalate, in the drinking water (*120*).

Carter et al. (*136*) examined the time course of DCA's effects in the liver of $B6C3F_1$ mice at concentrations of 0.5 and 5 g/L in drinking water. As reported in prior studies, the high-dose group displayed severe liver hypertrophy. However, a smaller but consistent increase in liver weight became evident with as little as 10 days of treatment at 0.5 g/L. Even at this relatively low dose, some hepatocytes appeared to have lost nuclei or possess nuclei that had undergone some degree of karyolysis. These experiments also appeared to rule out cytotoxicity and reparative hyperplasia as a consistent feature of DCA's effects.

Mather et al. (*140*) found increases in liver weight in rats treated with DCA at 5 g/L in their drinking water for 90 days. Relative liver and kidney weight to body weight were increased at concentrations of 0.5 g/L and above. PAS staining of liver sections revealed accumulation of PAS in severely swollen hepatocytes that was quite marked with 5 g/L DCA.

In contrast, DeAngelo et al. (*91*) reported that liver weights were not increased in male F344 rats chronically administered DCA. This conclusion is inconsistent with reports in other strains of rat. However, the design of the study may have tended to mask the hepatomegaly. The high dose of DCA was adjusted downward (from 2.6 to 1 g/L) after 18 weeks of treatment to avoid signs of overt toxicity in the rat. The earlier treatment clearly resulted in substantial losses in body weight that were apparently not recovered over the 84 weeks of the study. There were significantly elevated liver-to-body weight ratios in this group and an increase in both absolute and relative liver weights at the next lowest dosing schedule (0.5 g/L), the level associated with induction of liver tumors in a 2-year bioassay (*91*).

The dog appears to be very sensitive to the effects of DCA on the liver. Cicmanec et al. (*141*) examined the subchronic effects of DCA in dogs. Dogs were administered 12.5, 39.5, and 72 mg/kg/day for 90 days to groups of five males and five females. Liver weights were significantly increased in a dose-related manner beginning with the lowest dose. By comparison, the lowest effect level noted in mice is at 0.5 g/L of drinking water, which approximates 70–100 mg/kg per day, while the lowest effect level appears to be 125 mg/kg in rats (*122*). Thus, the hepatomegaly induced by DCA is consistently observed across species.

Comparing the Tumors Induced by DCA and TCA

Hyperplastic nodules and tumors induced by DCA have some characteristics that distinguish them from nodules and tumors that are induced by TCA. Pereira (*74*) indicated that in female mice both hepatocellular adenomas and carcinomas induced by DCA tended to be eosinophilic, whereas those induced by TCA were basophilic. In male $B6C3F_1$ mice treated with 2 g DCA/L, a substantial fraction (66%) of the AHF and nodules were reported to be eosinophilic (*117*). However, the larger lesions tend to be basophilic. These larger lesions included hyperplastic nodules, adenomas, and carcinomas. These data suggest that there are some differences in tumor induction by DCA based on sex. However, this difference appears to be important primarily at high doses (≥ 2 g/L) where the rate of cell replication is enhanced in a set of basophilic lesions. The development of these lesions may account for the much shorter latencies observed in male vs female mice at high doses (*73*).

As pointed out by previous investigators examining responses in male mice (*73,75*), Pereira et al. (*74*) found that the slope of the dose–response curves describing the induction of total lesions by DCA increases disproportionately with dose in female mice. A very

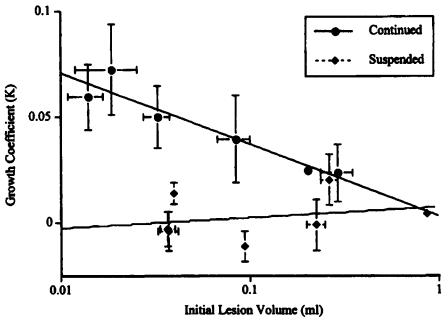

Figure 8. Effect of suspending DCA treatment on tumor growth rates. This experiment involved treatment of mice with 2 g/L of DCA until small tumors of approximately 1 mm diameter were detected with magnetic resonance imaging. Half the tumor-bearing animals were placed on distilled water, whereas the other half were maintained on the 2-g/L treatment. Tumors were re-imaged over the next 2–3 weeks. The growth rates are expressed as a coefficient of their initial volume. The vertical bars indicate the standard deviation of the differences in estimate of fractional growth, whereas the horizontal bars reflect the standard deviation of estimates of the differences in volume measurements. Data from Miller et al. (*138*).

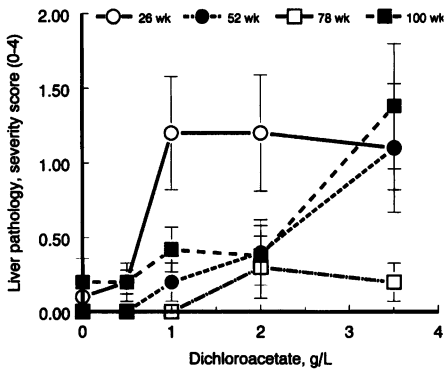

Figure 9. Severity of necrotic damage to the liver in male $B6C3F_1$ mice treated with various doses of DCA for different periods of a lifetime experiment. Note that such damage varies significantly in time but is only observed at doses of 1 g DCA/L drinking water and above. The necrosis involves limited portions of the liver (acinar necrosis). Data are adapted from DeAngelo et al. (*76*), but are typical of experiences in other laboratories (*119,120*). Each value represents the mean of scores in the livers of 10 mice ± SEM.

similar nonlinear relationship was observed in the initiation–promotion study (*113*). Conversely, the carcinogenic responses to TCA are essentially linear with dose in both types of studies. In part, the nonlinear character of the DCA dose–response area is a function of auto-inhibition of its metabolism as dose increases (*47,142*).

Stauber and Bull (*117*) found that DCA-induced liver tumors in male $B6C3F_1$ mice were immunoreactive to c-*Jun* and c-*Fos* antibodies, whereas TCA-induced liver tumors were not. It was found that this difference in phenotype could be duplicated by stimulating the growth of colonies from mouse hepatocytes suspensions on soft agar in the presence of the two compounds *in vitro* (*119*).

Latendresse and Pereira (*143*) also noted a number of differences in the phenotype of tumors promoted by TCA and DCA in the livers of female $B6C3F_1$ mice initiated with *N*-methyl-*N*-nitrosourea. These authors found that the majority of lesions promoted by DCA stained positively for TGF-α and glutathione *S*-transferase pi (GST_π). These markers were not observed in TCA-promoted lesions. Most interesting was the finding that DCA-promoted tumors expressed two cytochrome P450s not observed in the TCA-promoted tumors, CYP2E1 and CYP4A1. CYP2E1 is very important to the initial metabolism of TCE, particularly at low doses (*7*), indicating that metabolites would be produced in the target cell. This may be of particular importance to DCA-induced tumors.

In passing, it should be noted that the consistency of c-*Jun* expression noted in DCA-promoted liver tumors in MNU-initiated mice (*143*) was much lower than previously reported in tumors induced in male mice (*117*). This could be a sex difference, but it is important to recognize that different antibodies were used in these two studies. Antibodies to c-*Jun* typically have been raised against different phosphorylation sites in the DNA binding or transactivation regions of the c-*Jun* protein. These sites are likely to be differentially affected by c-*Jun* activation and, as a consequence, not too much emphasis should be placed on these experimental inconsistencies until there has been some resolution of this problem.

DCA- and TCA-promoted tumors differ in other ways. Tao and co-workers (*144*) report that cytosine methylation was depressed in normal liver and hepatic tumors initiated by MNU during promotion with either chemical. Cessation of treatment with DCA resulted in a return of cytosine methylation in adenomas to those found in noninvolved liver and that of control animals. This response was not reversed when TCA treatment was discontinued.

The easiest explanation for these differences is that DCA and TCA selectively modify the growth rates of different clones of cells that are present in the mouse liver. It was shown that treatment of normal hepatocytes by the two compounds had no effect on these phenotypes. It is difficult to explain such consistent differences on the basis of a genotoxic mode of action. These differences are much more easily explained by suggesting that the two chemicals interact with differing cell signal pathways in liver cells that already possess a modified genotype that renders them more sensitive to one compound or the other.

Probability That TCE Is Hepatocarcinogenic in Humans

The assessment of whether TCE poses a risk of liver cancer to humans first requires consideration of the available epidemiological literature. To the extent that epidemiological data are less than conclusive, the analysis must focus on the available data in experimental animals. To extrapolate with confidence, it is necessary to understand how the tumors are produced in the sensitive species and whether humans share a similar sensitivity. It is apparent from considerations above that both TCA and DCA have properties associated with tumor promotion. However, there are distinct differences in the types of lesions that they induce. One metabolite (TCA) appears capable of inducing tumors in only a single species, whereas the other (DCA) produces tumors in both rats and mice. The early effects of DCA on the liver of the dog suggest that this species may be even more sensitive to low-dose effects of this metabolite. Therefore, some further understanding of the mechanisms by which TCA and DCA act is necessary if TCE's potential to induce liver cancer in humans is to be determined with certainty. At this stage, deciding whether humans are sensitive is not based on strict comparisons of data generated in human versus test animal systems (*in vivo* or *in vitro*). Thus, at best a series of testable hypotheses (discussed below) can be suggested. Essentially, these reduce to the development of appropriate pharmacodynamic parameters that can be applied to measuring the relative intrinsic sensitivity of human livers in *in vitro*. Then pharmacokinetic models can be applied to account for kinetic and metabolic similarities and differences between species and across dose ranges. This would allow the probability that humans are at risk from TCE to be more precisely defined.

Simplistically, determination of the mode by which TCE induces liver tumors in mice must first consider: *a*) the effects that have been correlated with tumorigenesis by TCE, *b*) the extent to which these effects are accounted for by particular metabolites, and *c*) what levels of the particular metabolite must be achieved within the liver to produce their effects. A key finding that can be exploited is the fact that hepatic tumors induced by DCA can be distinguished from those induced by TCA; this can then be extended to identifying which of these characteristics are found in tumors induced by TCE.

Effects of TCE Associated with Liver Tumor Induction

For the purposes of this review, it may be concluded that there is no evidence that clearly associates a genotoxic effect of TCE or its hepatocarcinogenic metabolites with the induction of liver cancer. Essentially, this conclusion is based upon the weak mutagenic activity of the metabolites relative to the concentrations that are produced in the metabolism of TCE *in vivo* and evidence that other mechanisms are operative at these systemic concentrations.

Among the metabolites of TCE that are capable of inducing liver tumors, only CH has produced consistent genotoxic effects. These have been clastogenic rather than point mutations. The likelihood that CH contributes as a genotoxin to the carcinogenic effects of TCE appears remote. The rapid metabolism of this compound limits its concentrations to almost nonmeasurable levels, even in mice. Mice inhaling 600 ppm TCE have blood levels of 1.22 µg/ml (*145*), whereas approximately 150 µg CH/ml are observed with intravenous doses of 100 mg/kg of CH (*146*). While not directly comparable, these data suggest that there is a wide disparity in blood levels of CH when TCE is administered than when CH is administered directly. As a consequence, it seems more probable that the metabolites DCA and/or TCA are responsible for the carcinogenic effects of TCE.

Elcombe et al. (*67*) demonstrated that administration of TCE in a manner similar to that used in cancer bioassays of mice was capable of producing a sustained level of peroxisome proliferation. TCA is formed in sufficient concentrations to account for this response. It must also be concluded that TCA is making a significant contribution to the responses to TCE under the conditions of its cancer bioassays. Thus, TCA is probably making a substantive contribution to the induction of liver tumors in mice chronically treated with TCE. TCA also induced peroxisome proliferation in rats. However, neither TCA nor TCE induces liver tumors in rats.

Cytotoxicity and reparative hyperplasia are not prominent features of doses of TCE that are sufficient to induce cancer. At least with short-term treatment, TCE and two of

its metabolites modify cell replication and death rates. Increases in cell replication early in the course of treatment have been noted for TCE and appear to reflect a mitogenic effect (*67,68*). Stimulatory effects have also been reported for DCA at 2 and 2.6 g/L and TCA at concentrations in drinking water of 0.32–3.2 g/L (*117,121*). Almost all investigators have observed a depression of hepatocyte replication with continued treatment with both compounds (*121,137*).

It is remarkable how well the effects of DCA and TCA on cell division conform with their respective dose–response curves for cancer induction. The nonlinear relationships with dose in DCA-induced liver tumorigenesis, whether observed when bioassayed as a complete carcinogen (*73,74*) or as a promoter in an initiation–promotion study (*113*), are consistent with the differential inhibition of normal cell replication at low doses and the marked stimulation of replication within tumors at high doses (*117*). Conversely, the apparent linear dose–response curve with TCA-induced cancer under similar circumstances (*73,74,113*) is consistent with the fact that only the differential suppression of normal cell replication was observed (*117*). While the mechanisms of action of these two chemicals appear to be distinct, these data suggest that both are acting primarily through modification of cell birth and death processes.

The principal difference in the mechanisms of DCA and TCA appears to be related to the cell populations to which they provide a selective advantage. The tumors induced by TCA appear to have an innately greater rate of replication than those induced by DCA. In other words, TCA appears to be selecting initiated cells that are more aggressive than those selected by DCA at low doses. However, at higher doses the strong and selective mitogenic effect of DCA on tumor cells increases the growth rate of tumors with a less malignant phenotype (*114*). This stimulus to the growth rate is what appears to account for the very nonlinear dose–response relationship for tumorigenesis induced by DCA. The very large increase in tumor multiplicity in mice treated with DCA when doses are only increased by a factor of 2 in the high dose range that has been noted by all investigators reinforces this interpretation.

The extent to which replication rates in normal hepatocytes are decreased with longer term treatments with TCE has not been investigated. In itself, this possibility would not differentiate between the mechanisms postulated for DCA and TCA. A more diagnostic determination would be whether the tumors induced by TCE are sensitive to stimulation of cell replication rates by DCA. A second difference is the distinctly different phenotypes with respect to c-*Jun* immunoreactivity.

The inevitable confounding of rates of cell replication with rates of cell death makes it difficult to determine if the effects are exerted on one process or the other. The rate of cell replication must equal the cell death rate at steady state in normal tissue. The question of whether depressed cell replication rates within initiated cell populations result from downregulation in response to a chronic mitogenic effect or by a more specific interference with apoptosis can have substantively different implications for risk assessment. Depressed apoptosis could imply interference with the death of a cell in which damage to DNA has been recognized by the cell. The replication of such cells could be an efficient means of producing initiated cells. Until such time that these cells progress to more stable lesions, they should be very sensitive to apoptosis if treatment is suspended. However, if cells are selected that have a higher probability for progression to tumor, then rapid induction of a malignant phenotype might be observed. Conversely, a simple downregulation of mitogenesis may be a more benign process. Such differences may underlie the apparently less aggressive properties of DCA-induced tumors rather than TCA-induced tumors (*73,114*).

While the mechanisms underlying these modifications of cell division and death are not yet known, these findings frame a mode of action for TCE-induced liver cancer that is not dependent upon chemically induced mutation. How are these findings to be translated into an assessment of potential human risks to liver cancer induced by TCE? Most critical to the low-dose extrapolation is whether small disturbances in cellular control mechanisms have pathological consequences. If DCA- and TCA-induced mechanisms can be assumed to be good models for TCE-induced carcinogenesis, it would appear that tumors are only induced by doses of the compounds that result in significant downregulation of normal control mechanisms in normal cells. Apparently it is this negative selection process that is active at low doses of both metabolites. If sufficient perturbation has to be produced in normal cells for downregulation to be observed, then it is probable that the tumorigenic response has an effective threshold.

DCA and TCA both produce hepatomegaly in the dose ranges in which they induce hepatic cancer. In part, these changes reflect the initial effects of both chemicals on increasing cell replication rates. However, some of the effects of both compounds, but most particularly DCA in mice, are the results of cytomegaly. Empirically, it might be useful to consider TCE's effects on liver weight as an early measure of processes that may be involved in liver cancer induction. The substantive differences in the character of the liver enlargement that is induced by the two metabolites indicate that care must be taken not to carry this parallel too far.

It is clear from this analysis that there is altered control of cell replication in TCE-treated mice. These effects have been demonstrated by administering either TCE directly or one of two metabolites thought responsible for its induction of liver tumors in mice. Mutation spectra in codon 61 of the H-*ras* protooncogene are consistent with a role of DCA in TCE-induced liver cancer. It is unlikely that these mutations play a significant role in the initiation of the tumorigenic response to either TCE or DCA because these mutations are generally less abundant than the wild-type form of the gene in most tumors and therefore would not be an early event in the process (*42,45*).

Effective Levels of TCE Metabolites

Fisher (*70*) has provided a review of the pharmacokinetics of TCE and its metabolites. It is necessary, however, to touch on this issue briefly to support any conclusions that a particular metabolite can be held completely or partially responsible for liver tumor induction.

TCA is the metabolite that circulates in the blood at the highest concentration in all species, including humans (*81,83,145,146*). Under conditions of the bioassays for TCE, it appears that there is evidence of peroxisome proliferation in mice (*67*). Blood concentrations of TCA following administration of doses of TCE used in the NCI and NTP studies are in the same range required for activation of the PPARα (*147*). Thus, TCA can account for these observations with TCE.

The other hepatocarcinogenic metabolite is DCA, which could arise through several pathways (*54,148–150*). Recent data indicate that at carcinogenic doses of TCE, DCA in blood can be barely detected but not quanitified, and then only after its metabolism was inhibited by prior treatment (*48*). Thus, an upper limit of 1–2 μM (130–260 ng/mL) can be estimated as a maximum concentration of DCA following a 1-g/kg dose of TCE administered by gavage. A more probable concentration would be about an order of magnitude lower based on the clearance of a 20-mg/kg dose of DCA from the blood of naïve $B6C3F_1$ mice (*48*). In contrast, concentrations of DCA in blood of mice chronically treated with 2 g DCA/L were found to be 300 μM (39 μg/ml) during the period when they were actively drinking water (5 A.M.), but fall to 10% of this level 4 hr later (9 A.M.). Concentrations observed with DCA treatment at 0.5 g/L were found to be approximately 2–4 μM (250–500 ng/mL) during the night (*47*), but these concentrations fell rapidly to levels below the limit of

quantitation by 9 A.M. Thus, the concentrations of DCA in the blood of mice treated with 1,000 mg/kg TCE (2 μM) may be within range of a concentration that appears effective in producing liver cancer in mice treated with 0.5 g/L DCA directly. These concentrations are within an order of magnitude of those that produced clonal expansion of cells sensitive to DCA *in vitro* (20 μM) (*119*). Other more complex mechanisms may be active in the intact animal, possibly an indirect mechanism that stems from differential accumulation of glycogen in normal liver cells and liver tumor cells (*73*). This type of mechanism would require interactions between the initiated cells and adaptation of serum hormone levels to effects produced in normal hepatocytes. Modeling such complex responses has not been attempted in an *in vitro* system. The complex differences that result from differing modes of DCA and TCE administration make further comparisons difficult without additional data. However, TCE still may be metabolized to DCA in quantities that it could contribute to the carcinogenic response.

DCA is unlikely to contribute to the induction of peroxisome synthesis at levels that are produced by the metabolism of TCE. Maloney and Waxman (*147*) found that concentrations in the range of 0.5–1 mM are necessary to activate the PPARα. These concentrations are about three orders of magnitude higher than would be produced from the metabolism of TCE and the concentrations of DCA that result from the direct administration of DCA at doses that are clearly carcinogenic in the mouse.

A direct role for CH in the induction of liver tumors is difficult to establish because it is largely converted to TCA *in vivo* (*146*). A possibility of it making an independent contribution to hepatocarcinogenicity of TCE is of concern because, as discussed above, this metabolite appears to be clastogenic. The vanishingly small concentrations of CH that would be anticipated from environmental exposures to TCE relative to those that arise from carcinogenic doses of CH that is directly administered, however, make this contribution unlikely.

Since TCE, CH, and TCA do not induce liver tumors in rats, it is difficult to support the hypothesis that they represent a transspecies risk for this tumor site. Only DCA would appear to have the capability of inducing liver cancer in multiple species. Recent data suggest that DCA's contribution to the induction of liver cancer in humans by low doses of TCE would be negligible. That is because of the lower formation DCA anticipated in humans relative to mice and the probability that it will have a sublinear toxicodynamic profile.

Implications for Assessment of Risks at Low Doses

The available data suggest that the induction of liver tumors by TCE results from a modification of cell-signaling systems that control rates of cell division and death. Unlike chemicals that induce mutation, such mechanisms have only been recently dealt with in models for low-dose extrapolation. In no case have the risks for such chemicals been explicitly dealt with in the regulatory arena.

In the case of TCE, the issue is complicated by several factors: *a*) It is probable that more than one cell-signaling system is being modified, at least one by TCA and another by DCA. *b*) The details of these pathways are not completely known, and it will be difficult, based upon currently available data, to predict how simultaneous modification of these pathways might alter the outcome. *c*) It is not possible to describe their control functions in detail and in quantitative terms. *d*) Although critical components of both pathways are found in most mammalian species, there are clearly differences in the amount of receptor present and the overall mechanisms of regulation in different species with at least one of these pathways (i.e., PPARα-dependent signaling).

Cell signaling falls into two general types: Those linked to receptor proteins that are embedded in the cell membrane (e.g., epidermal growth factor, insulinlike growth factors) and those that are intracellular receptors (e.g., estrogen receptors, PPARs) (*151*). The pathways coupled to membrane receptors typically involve a cascade of protein–protein interactions that involve cycles of phosphorylation/dephosphorylation of key intermediate signaling proteins. There are often several different phosphorylation sites on these proteins and the functional state of the protein can be modified independently and in opposite directions by phosphorylation at different sites. The nuclear receptors generally fall into a category of ligand-activated transcription factors. Control of the transcription of particular proteins can be modified through formation of a variety of heterodimers that facilitate or inhibit the transcriptional activation of the complex. Frequently, the activity of these factors can be modified by phosphorylation/dephosphorylation cycles.

Both membrane receptor and intracellular receptor signaling pathways have complex functions. Frequently, similar functional activities can be influenced by both types of pathways and by a variety of pathways within each group. For example, apoptosis can be initiated by stimulation of T-cell, *Fas/Apo1*, steroid, TGF-β, or tumor necrosis factor receptors (*152*). Both types of cell-signaling systems are activated or inhibited by a wide variety of physiological variables, as well as being modified by chemicals that produce cancer. Both are known to influence rates of cell division and death. Moreover, they modify one another's activities in this respect. A second example is that insulin induces phosphorylation of the PPARα and increases its transcriptional activity (*153*). At present, the unknowns involved in each type of control mechanism are too many to incorporate formally into models for low-dose extrapolation. Therefore, it is important to develop more general conceptualizations of how such modes of action are likely to contribute to carcinogenic responses at low doses.

As a result of our limited knowledge, it is easier to analyze how perturbations in these control systems manifest themselves in processes known to affect the course of carcinogenesis. Carcinogenic effects would result from a chemical's ability to activate a pathway that increases cell replication or to inhibit a pathway that activates apoptosis. Apoptosis and cell replication are not independent variables and, in part, make use of some of the same signaling pathways (*154*). In mature organisms, cell birth and cell death rates are equivalent under normal circumstances. A carcinogen that acts by modifying these processes can do so by providing a clone of initiated cells a growth advantage relative to normal cells (*154,155*). There are implications for low-dose extrapolation that relate to a more rapid rate at which cells are turning over within a tissue, as this appears to lead to increased mutation rates (*156*).

In general, if the primary molecular interaction of a chemical is with a protein involved in cell signaling, the impact at low doses may differ depending upon where signal transduction within the pathway is modified. Typically, these systems amplify signals, so the impact of interference with a receptor molecule at the cell membrane or at the level of the regulated transcription factor may have differing dynamics than interference with some intermediate step in a signaling cascade. The implications for low-dose extrapolation may also differ depending upon whether the chemical is directly affecting the critical pathway or is the result of cross-talk from parallel pathways with another set of regulatory functions. In addition, all such pathways are subject to downregulation by one means or another. A strong, sustained downregulation means that the normal cells have lost the effective control provided by that signaling pathway. The cell then must depend on other systems to control the primary functions of the downregulated pathway. Inevitably this leads to some imbalances that may produce other disturbances in homeostatic mechanisms that range from basic control of intermediary metabolism to

higher level organ-specific functions. This becomes analogous to mutation, where initiated cells can no longer make effective use of a pathway controlling cell replication or death.

To a point, the dynamics of a cell-signaling pathway are based on the classical continuous or graded dose–response curves that are familiar to pharmacologists. At some stage the activation or inhibition of such a pathway results in a stochastic response, i.e., an additional cell division or prevention of the death of a single cell. In other areas of biology, this behavior is viewed as a threshold. Are there thresholds that limit the impact of minor perturbations in the cell-signaling systems that control birth and death rates of cells? This question has received little experimental attention in carcinogenesis.

Another critical question is reversibility. It is reasonably clear that within the physiological range, modification of cell-signaling pathways by chemicals should be reversible, either through dissociation of the chemical or by biosynthetic replacement of an irreversibly damaged protein. Even stochastic responses such as cell replication can be reversed by early apoptosis. As these responses become sustained, however, some irreversible pathological events (such as immortalization) may be triggered. In the case of increased cell replication, it has been held that each cell division carries with it a probability of error in DNA replication (*156*). The destiny of these mutated cells is known to depend on a number of factors too numerous to deal with here. However, it is clear from animal studies that while many cells become initiated, the probability that they will grow and progress to tumor is small (*157*). Conversely, we know from human experience that such rare events underlie the development of cancer (*158*). The question is whether mutation rate is really the rate-limiting process. Survival and replication of initiated cells may actually be more important with some environmental carcinogens.

In a similar fashion, the impact of suppressed apoptosis may be short-lived if exposure is intermittent. What is the fate of a stem cell whose death has been temporarily postponed? Are such cells likely to die when the chemical exposure is intermittent or is terminated? Evidence for such a fate seems to be coming from stop experiments, in which tumors are observed to regress after removal of the chemical treatment. Regressions of this type are well established in cancer resulting from initiation–promotion studies in the skin (*159*), and there are recent examples of such behavior in liver tumor induction as well (*160*). It is difficult to envision a linear dose–response curve at low doses if the response remains reversible at such a late stage in tumorigenesis.

As stated earlier, there must be some differential effect of a chemical on normal cells and initiated cells for it to be an effective promoter of cancer. It is probable that such differential sensitivity will arise from a lessor ability of the initiated cell to downregulate the system responding to the chemical treatment. To some extent this appears to be characteristic of both DCA and TCA, despite the fact that they tend to stimulate growth of tumors with different characteristics. From the work of Stauber and Bull (*117*), the initial stimulation of cell replication within the normal cell population appears to be followed not only by a loss of sensitivity to the mitogenic effects of the compound but also by an actual inhibition of cell replication. In neither case was the rate of replication among initiated cells so reduced. In the case of DCA, the initiated cells contained within nodules or tumors appear to retain their sensitivity to the mitogenic effects of higher doses, but the downregulation of normal cell replication appeared to be the predominant effect at lower doses. This general mode of action has been associated with other tumor promoters such as phenobarbital (*118*). A critical question that arises from this observation is whether there is any increased risk for cancer from chemicals that act this way at doses below those that result in downregulation of the mitogenic response. If increased risk could be established, then there would be a clear basis for considering threshold exposure levels for tumor-promoting agents. If this can be shown, the next question is how to experimentally identify the doses at which this occurs.

For experimentalists, the critical question is whether the probability of a tumorigenic response can be quantitatively associated with a transition between a physiologically responsive pathway, one that has been downregulated to the point that it is no longer responsive to normal variations in physiological conditions. There is a concordance in the dosing schedules of DCA and TCA that produce downregulation of a mitogenic effect of the chemicals on normal cells and those that produce hepatic tumors in mice (*71,113, 117,137*). While this dosing level with DCA produces > 80% incidence of tumors in mice over a lifetime, there is no evidence of carcinogenic response at dosing levels one order of magnitude lower (*71,76*). To the extent these two metabolites can account quantitatively and qualitatively for liver tumors induced by TCE in mice, the risk for liver cancer should approach zero at doses of TCE that do not generate sufficient amounts of these metabolites to induce these responses.

A number of expert panels (*1,52*) have reviewed the risk assessment issues with peroxisome proliferators and have concluded that there is insufficient basis for concluding that these compounds do not represent a carcinogenic hazard to humans. However, both panels have generally concluded that peroxisome proliferators, per se, are unlikely to represent a carcinogenic hazard under anticipated conditions and levels of exposure, but their carcinogenic potential cannot be ruled out under any conditions of exposure. Cattley et al. (*52*) suggested that a case-by-case assessment using a margin-of-exposure approach would be most appropriate for attempting to provide quantitative estimates of risk. The panel also concluded that all available data be considered, in part because an ability to act as a peroxisome proliferator does not exclude the possibility of other properties that could independently contribute to the development of cancer.

Research Questions Remaining

When changes occur in the guidelines for risk assessment such as those recently proposed by U.S. EPA (*4*) that allow mechanistic data to substantively impact the weight-of-evidence and quantitative evaluations, questions arise as to the conclusive nature of the data. It will be a rare circumstance, at least in the foreseeable future, if the mode of action of many carcinogens is fully described with confidence. The question is really defining when the available data are sufficient for moving beyond the default positions in a risk assessment. Research to further clarify the issue could go on endlessly. If that appears to be necessary, however, one must seriously question the role that new science should have in the decision-making process. The following listing of research areas relative to clarifying the mode of action of TCE (and its metabolites) in the induction of liver cancer represents a finite series of questions that would appear more-or-less critical to near-term decision-making. However, the list is not meant to suggest that all research is absolutely essential before a change in risk policy for TCE can be made.

Clarification of the Role of Metabolites in the Hepatocarcinogenic Effects of TCE

Defining the nature of interactions between CH, DCA, and TCA in liver tumor induction in mice would aid in a better understanding of how the tumorigenic activity of TCE should be extrapolated to low dose. To be useful, these studies would have to be quantitative to ensure that the concentrations produced *in vivo* are realistic with respect to the expected concentrations seen within the liver.

Relevance of the Mechanisms Involved in Hepatic Carcinogenesis of TCE to Humans

The result of activating the PPARs in human systems is becoming clearer, but the connection

of those responses to the tumorigenic responses in rodents requires further delineation. At present, data with regard to TCA identify it as a peroxisome proliferator, but this evidence is correlative and far from definitive. Experiments with PPARα-Knockout mice could provide more definitive evidence.

In the case of DCA, understanding the effects of chronic TCE-treatment on serum insulin levels and normal hepatocyte and liver tumor cell levels of insulin receptor expression would be useful in determining the extent to which DCA plays a role in the induction of liver tumors. Clearer delineation of perturbations induced by either metabolite in cell-signaling pathways will allow the direct measures of human responses to be evaluated. These biomarkers of effect could greatly facilitate evaluation of whether humans are at significant risk of liver cancer from TCE and several other related solvents. In the case of both TCA and DCA, there is a clear path for comparing human and rodent sensitivities to these metabolites of TCE using the *in vitro* clonal expansion system described by Stauber et al. (*117*).

Behavior of the Mechanisms of Liver Tumor Induction at Low Doses

One must continually keep in mind that evidence of tumorigenic response to TCE and its metabolites has been obtained only at very high doses. Virtually all of the concern that is generated about the low levels of TCE that are encountered in the general environment come from the application of a linear extrapolation model to what appear to be strain-specific responses obtained at these very high doses. The use of the linear model for dose–response modeling is based upon assumptions about a genotoxic mode of action of TCE and its metabolites in the induction of liver cancer. Data have emerged that indicate that this assumption does not apply. Evidence is growing that DCA and TCA act primarily by modifying cell-signaling systems. Since the activity of cell-signaling systems is constantly modified as part of normal physiological function, it seems unlikely that perturbations that do not take them outside their normal operating limits are going to have irreversible and cumulative effects. This hypothesis is testable using variations in the clonal expansion system used by Stauber et al. (*117*) and by *in vivo* experiments that more precisely pursue the effects of TCE's metabolites on rates of cell division and cell death within initiated cell populations at low doses.

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